Fecal Coliform TMDL for Christians Creek, Augusta County, Virginia

Submitted by

Virginia Department of Environmental Quality
Virginia Department of Conservation and Recreation

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1. Background

Section 303(d) of the Clean Water Act and EPA's Water Quality Planning and Management Regulations (40 CFR Part 130) require states to develop Total Maximum Daily Loads (TMDLs) for waterbodies which are exceeding water quality standards. TMDLs represent the total pollutant loading that a waterbody can receive without violating water quality standards. The TMDL process establishes the allowable loadings of pollutants for a waterbody based on the relationship between pollution sources and in-stream water quality conditions. By following the TMDL process, states can establish water quality based controls to reduce pollution from both point and nonpoint sources to restore and maintain the quality of their water resources (EPA, 1991).

2. Impairment Listing

Christians Creek (Virginia waterbody ID VAV-B14R) was listed as impaired on Virginia's 1998 303(d) Total Maximum Daily Load Priority List and Report (VADEQ, 1998) due to violations of the State's water quality standard for fecal coliform at two monitoring stations, and for violations of the General Standard (benthics). On March 11, 2002, VADEQ requested EPA to remove the Christians Creek segment previously listed as having a benthic impairment from the 1998 303(d) list because recent data indicate significant improvement in the aquatic life community. This report addresses only the fecal coliform impairment. Out of 55 fecal coliform samples collected during the 1998 assessment period at the Rte. 794 bridge (river mile 12.32), 21 violated the water quality standard. During the subsequent 2000 assessment period, 19 of 57 samples violated the water quality standard. Out of 48 samples collected during the 1998 assessment period at the Rte. 831 bridge (river mile 21.76), 30 violated the water quality standard. During the subsequent 2000 assessment period, 31 of 56 samples violated the water quality standard.

Christians Creek is located in Augusta County and is part of the Shenandoah River Basin (USGS Hydrologic Unit Code 02070005). The impaired segment has the segment ID VAV-B14R-01 and is 31.52 miles in length. It begins at the headwaters and continues downstream to its confluence with the Middle River.

3. Applicable Water Quality Standard

According to Virginia Water Quality Standards (9 VAC 25-260-5), the term *"water quality standards means provisions of state or federal law which consist of a designated use or uses for the waters of the Commonwealth and water quality criteria for such waters based upon such uses. Water quality standards are to protect the public health or welfare, enhance the quality of water and serve the purposes of the State Water Control Law (§62.1-44.2 et seq. of the Code of Virginia) and the federal Clean Water Act (33 USC §1251 et seq.)."*

3.1. Designated Uses

According to Virginia Water Quality Standards (9 VAC 25-260-10A), *"all state waters are designated for the following uses: recreational uses (e.g., swimming and boating); the propagation and growth of a balanced indigenous population of aquatic life, including game fish, which might be reasonably expected to inhabit them; wildlife; and the production of edible and marketable natural resources (e.g., fish and shellfish)."*

3.2. Applicable Water Quality Criteria

For a non-shellfish supporting waterbody to be in compliance with Virginia fecal coliform standards for contact recreational use, VADEQ specifies the following criteria (9 VAC 25-260-170):

> *"…the fecal coliform bacteria shall not exceed a geometric mean of 200 fecal coliform bacteria per 100 mL of water for two or more samples over a 30-day period, or a fecal coliform bacteria level of 1,000 per 100 mL at any time."*

If the waterbody exceeds either criterion more than 10% of the time, the waterbody is classified as impaired and a TMDL must be developed and implemented to bring the waterbody into compliance with the water quality criterion. Based on the sampling frequency, only one criterion is applied to a particular datum or data set (9 VAC 25-260-170). If the sampling frequency is one sample or less per 30 days, the instantaneous criterion is applied; for a higher sampling frequency, the geometric mean criterion is applied.

For Christians Creek, the TMDL is required to meet the geometric mean criterion since the computer simulation gives daily fecal coliform concentrations, analogous to daily sample collection. The TMDL development process also must account for seasonal and annual variations in precipitation, flow, land-use, and pollutant contributions. Such an approach ensures that TMDLs, when implemented, do not result in violations under a wide variety of scenarios that affect fecal coliform loading.

3.3. Water Quality Standards Review

Two regulatory actions related to the fecal coliform water quality standard are currently under way in Virginia. The first rulemaking pertains to the indicator species used to measure bacteria pollution. The second rulemaking is an evaluation of the designated uses as part of the state's triennial review of its water quality standards.

<u>Indicator Species</u>

EPA has recommended that all States adopt an *E. coli* or enterococci standard for fresh water and enterococci criteria for marine waters by 2003. EPA is pursuing the States' adoption of these standards because there is a stronger correlation between the concentration of these organisms (*E. coli* and enterococci) and the incidence of gastrointestinal illness than with fecal coliform. *E. coli* and enterococci are both bacteriological organisms that can be found in the intestinal tract of warm-blooded animals. Like fecal coliform bacteria, these organisms indicate the presence of fecal contamination. In Virginia, the adoption of the *E. coli* and enterococci standard is scheduled for 2002.

<u>Designated Uses</u>

All waters in the Commonwealth have been designated as "primary contact" for the swimming use regardless of size, depth, location, water quality or actual use. The fecal coliform bacteria standard as described in 9 VAC 25-260-170 and on page 2 above is to be met during all stream flow levels and was established to protect bathers from ingestion of potentially harmful bacteria. However, many headwater streams are small and shallow during base flow conditions when surface runoff has minimal influence on stream flow. Even in pools, these shallow streams do not allow full body immersion during periods of base flow. In larger streams, lack of public access often precludes the swimming use.

In the TMDL public participation process, the residents in these watersheds often report that " people do not swim in this stream." It is obvious that many streams within the state are not used for primary contact recreation.

Additionally, the VADEQ and VADCR have developed fecal coliform TMDLs for a number of impaired waters in the State. In some of the streams, fecal coliform bacteria counts contributed by wildlife result in standards violations, particularly during base flow conditions. Wildlife densities obtained from the Department of Game and Inland Fisheries and analysis or "typing" of the fecal coliform bacteria show that the high densities of muskrat, beaver, and waterfowl contribute to the elevated fecal bacteria counts in these streams.

Recognizing that all waters in the Commonwealth are not used extensively for swimming, Virginia is considering re-designation of the swimming use for secondary contact in cases of: 1) natural contamination by wildlife, 2) small stream size and 3) lack of accessibility to children. The widespread socio-economic impacts resulting from the cost of improving a stream to a "swimmable" status are also being considered.

The re-designation of the current swimming use in a stream to a secondary contact recreational use will require the completion of a Use Attainability Analysis (UAA). A UAA is a structured scientific assessment of the factors affecting the attainment of the use which may include physical, chemical, biological, and economic factors as described in the Federal Regulations. The stakeholders in the watershed, Virginia, and EPA will have an opportunity to comment on these special studies.

4. Source Assessment

4.1. Point and Nonpoint Sources

One of the major obstacles to improving stream water quality is that the potential sources of fecal coliform bacteria are numerous and the dominant sources are generally unknown. This makes it difficult to direct effective cleanup efforts. As part of their TMDL development study, the US Geological Survey (USGS) Virginia District investigated the concentrations of fecal coliform bacteria during different flow conditions and the sources of the fecal coliform bacteria that are impacting Christians Creek.

To find out how fecal coliform concentrations vary during different flow conditions, the USGS monitored fecal coliform bacteria at different locations in Christians Creek and under different flow conditions (high, low and intermediate flow events). Concentrations of fecal coliform bacteria were found to be smallest during low-flow periods, intermediate during recession-flow periods (after storms when the flow is decreasing), and highest during storm events. This suggests that the runoff-driven sources of pollution are the dominant factor in the Christians Creek impairment. The State's water-quality standard (1,000 colonies/100 mL) is usually met during low-flow periods.

The Christians Creek watershed contains both point and nonpoint sources of fecal coliform bacteria. Permitted point sources such as sewage treatment plants (STPs) can contribute bacteria loads to surface waters through continuous or intermittent effluent discharges. Nonpoint sources are diffuse sources that enter the stream during runoff events, by direct deposition into the stream (e.g. by cattle) and to some extent through the groundwater.

Point sources in Virginia are permitted under the Virginia Pollutant Discharge Elimination System (VPDES) program. Dischargers with discharges of less than or equal to 1,000 gallons per day are permitted under a VPDES general permit. Permitted point sources existing during TMDL development in Christians Creek are presented in table 1. There were six individual and twelve general permit holders in the watershed. Both the general and the individual permits specify a fecal coliform limit of 200 colonies/100mL in the effluent. The combined average waste load from all permitted facilities was 2.36×10^{12} colonies/year.

Two of the existing facilities, the Brookwood and Staunton (STPs), were removed from service in the summer of 2001, and the wastewater flows from these areas transferred to the Fishersville STP. After the TMDL was developed, the Augusta County Service Authority also informed VADEQ that an expansion from the current design flow of 2 mgd to 4 mgd was planned for the Fishersville STP by 2010. In order to accommodate this additional flow, an additional model run was generated at the new design capacity of 4 mgd. The new wasteload allocations are presented in table 2 below. The average discharge rate was used to simulate existing conditions (see table 1). The maximum permitted discharge rate was used to simulate the TMDL condition (see table 2). In both conditions, the maximum permitted fecal coliform concentration of 200 colonies/100mL was used. This approach provides an implicit margin of safety because chlorination at the waste water treatment plants typically results in fecal coliform concentrations of less that 50 colonies/100mL. The combined waste load allocation from all permitted facilities was 11.8 x 10^{12} colonies/year.

Table 1. Permitted point sources of fecal coliform bacteria in Christians Creek represented in model runs for existing conditions

Permit Number	Facility Name	Average Discharge Rate (gpd)	Fecal Coliform Permit Limit (colonies/100mL)	Average Waste Load (colonies/year)
VA0025291	Augusta Co. Service Authority	700,000	200	1,940 x 10^9
VA0022306	Augusta Co. Service Authority – Staunton	90,000	200	249 x 10^9
VA0022292	Augusta Co. Service Authority – Brookwood	30,000	200	83.2 x 10^9
VA0020427	Augusta Co. School Board	14,000	200	38.8 x 10^9
VA0089061	Woodlawn Village L.L. Corp.	7,000	200	19.4 x 10^9
VA0086738	Southern States Coop, Inc.	0	200	0
VAG401655	Single Family Home	1,000	200	2.76 x 10^9
VAG401967	Single Family Home	1,000	200	2.76 x 10^9
VAG401968	Single Family Home	1,000	200	2.76 x 10^9
VAG401082	Single Family Home	1,000	200	2.76 x 10^9
VAG401138	Single Family Home	1,000	200	2.76 x 10^9
VAG401159	Single Family Home	1,000	200	2.76 x 10^9
VAG401195	Single Family Home	1,000	200	2.76 x 10^9
VAG401203	Single Family Home	1,000	200	2.76 x 10^9
VAG401443	Single Family Home	1,000	200	2.76 x 10^9
VAG401449	Single Family Home	1,000	200	2.76 x 10^9
VAG401869	Single Family Home	1,000	200	2.76 x 10^9
VAG401969	Single Family Home	1,000	200	2.76 x 10^9
	TOTAL			2,364 x 10^9

Table 2. Updated inventory for permitted point sources of fecal coliform bacteria in Christians Creek represented in model runs for TMDL conditions

Permit Number	Facility Name	Design Flow Rate (gpd)	Fecal Coliform Permit Limit (colonies/100mL)	Waste Load Allocation (colonies/year)
VA0025291	Augusta Co. Service Authority – Fishersville expanded	4,000,000	200	$11,085.7 \times 10^9$
VA0090417	Augusta Co. Service Authority - Greenville	25,0000	200	690.0×10^9
VA0020427	Augusta Co. School Board	16,000	200	44.3×10^9
VA0089061	Woodlawn Village L.L. Corp.	15,000	200	41.6×10^9
VA0086738	Southern States Coop, Inc.	0	200	0
VAG401655	Single Family Home	1,000	200	2.76×10^9
VAG401967	Single Family Home	1,000	200	2.76×10^9
VAG401968	Single Family Home	1,000	200	2.76×10^9
VAG401082	Single Family Home	1,000	200	2.76×10^9
VAG401138	Single Family Home	1,000	200	2.76×10^9
VAG401159	Single Family Home	1,000	200	2.76×10^9
VAG401195	Single Family Home	1,000	200	2.76×10^9
VAG401203	Single Family Home	1,000	200	2.76×10^9
VAG401443	Single Family Home	1,000	200	2.76×10^9
VAG401449	Single Family Home	1,000	200	2.76×10^9
VAG401869	Single Family Home	1,000	200	2.76×10^9
VAG401969	Single Family Home	1,000	200	2.76×10^9
	TOTAL			$11,894.52 \times 10^9$

Nonpoint sources of bacteria that were identified and assessed in the Christians Creek watershed during TMDL development are human, dogs, cats, and a variety of wildlife species. For each of the sources, population numbers and fecal coliform production were calculated based on knowledge about the watershed and bacteria source tracking.

4.2. Bacteria Source Tracking

In order to further detail bacteria contributions to the stream, fecal coliform samples were also subjected to a genetic fingerprinting analysis known as ribotyping. This technique allows the identification of sources of the fecal coliform bacteria that are impairing Christians Creek. The ribotyping technique is actually performed on *E. coli* bacteria, which are the dominant members of the fecal coliform bacteria group. The ribotyping technique works because each warm-blooded animal species possess genetically unique *E. coli*, and ribotyping can characterize these genetic differences. The ribotyping analysis isolates and characterizes a specific portion of the *E. coli* DNA (the gene that codes for ribosomal RNA production); this specific gene is then compared to *E. coli* genes from known animals. A library of known *E. coli* fingerprints was developed by collecting and sampling the fecal waste (which contains species-specific *E. coli*) from the potentially contributing animal species such as dogs and geese.

The ribotyping results indicate that diverse sources contribute to the fecal coliform impairment of Christians Creek. The dominant sources are cattle, poultry, and human. Other sources identified through ribotyping include dog, horse, deer, cats, duck, goose and raccoons. The identification of poultry, cattle and humans as the three dominant fecal coliform sources in Christians Creek is reasonable based on the large populations of poultry, cattle and humans in the watershed.

Based on bacteria source tracking, data collection in the watershed and literature searches, the following sources of fecal coliform bacteria were represented in TMDL development.

Table 3a. Human, pet and wildlife sources of fecal coliform bacteria in the Christians Creek watershed

Source	Population (#)	Fecal Production (grams/day)	Fecal Coliform Density (colonies/gram)
Human[1]	1,562	150	466.0E+6
Dog[1]	28,474	450	4.1E+6
Cat[1]	75,640	20	14.9E+6
Deer	975	772	224.0E+6
Goose[1] **Summer/winter**	23,292/ 30,359	225	3.6E+6
Duck[1] **Summer/winter**	3,808/ 7,849	150	49.0E+6
Raccoon[1]	8,129	450	11.1E+6
Muskrat[1]	479	100	0.3E+6
Beaver[1]	10	200	0.001E+6

[1]Population equivalents based on model calibration

Table 3b. Livestock sources of fecal coliform bacteria in the Christians Creek watershed

Source	Population (#)	Fecal Production (grams/day)	Fecal Coliform Density (colonies/gram)
Dairy (P)^	210	54,545	0.2E+6
Dairy (C)	90	54,545	0.2E+6
Dairy (S)	<1	54,545	0.2E+6
Heifer (P)	299	39,091	1.4E+6
Heifer (S)	1	39,091	1.4E+6
Beef (P)	897	20,909	0.6E+6
Beef (S)	3	20,909	0.6E+6
Poultry	286,000	113	3,730.0E+6
Horses[1]	165	23,182	2.2E+6
Sheep[1]	175	1,091	1.8E+6

^ (P) = pasture, (C) = confinement, (S) = stream; cattle population distribution presented for June
[1]Population equivalents based on model calibration

5. TMDL Development

5.1. TMDL Allocation Scenarios

The Christians Creek fecal coliform bacteria TMDL was developed by the US Geological Survey (USGS) Virginia District using the Hydrologic Simulation Program – Fortran (HSPF). All sources of fecal coliform were considered during the development of the TMDL. The specific methodologies used during the TMDL development process are described in detail in Appendix A. Table 4 shows potential TMDL allocation scenarios for Christians Creek. All three scenarios result in 0% exceedance of the geometric mean standard with a 5% explicit margin of safety, i.e. the geometric mean does not exceed 190 cfu/100mL at any time. The 5% explicit margin of safety is in addition to the implicit margin of safety described in section 4.1. The last scenario is the selected TMDL scenario. This scenario is slightly different from the TMDL scenario proposed in the final public meeting because of the increased capacity simulated for the Fishersville STP (94% instead of 93% for all non-human sources on pervious surfaces). However, because of the implicit margin of safety discussed in section 4.1., it is not expected that these reductions will need to be implemented. The land-use based loading associated with scenario 3 is presented in Table 5.

Table 4. Allocation scenarios for Christians Creek TMDL

Scenario Number	**Percent reduction in loading from existing condition**						
	Impervious Land Surface	**In-Stream**	**Pervious Land Surface**				
	Dog	**Cattle/ Human**	**Human[1]**	**Pets**	**Cattle& Poultry**	**Sheep& Horses**	**Wildlife[2]**
1	90	100/100	100	100	100/100	100	0
2	100	98/100	100	100	100/100	100	50
3	**99**	**99/100**	**100**	**94**	**94/94**	**94**	**94**

[1]Represents the cumulative effect of failing septic systems, overflowing and leaking sewer lines and illicit connections

[2]Represents the reductions simulated for goose, duck and deer. Reductions for raccoon in scenario 3 are the same as shown for wildlife. No reductions were simulated for muskrat and beaver in scenarios 1-3 and for raccoon in scenario 1 and 2.

Table 5. Land-use based loadings to Christians Creek for TMDL Scenario 3

Land Use Category	Average Annual Loading for Current Conditions (col/year)	Average Annual Loading for TMDL Conditions (col/year)	Percent Reduction Required (%)
Urban	2.54E+15	101.0E+12	96.01
Residential	10.1E+15	123.0E+12	98.78
Pasture	11.5E+15	496.0E+12	95.69
Cropland	7.05E+15	428.0E+12	93.94
Hayland	4.17E+15	166.0E+12	96.01
Forest	1.38E+15	58.1E+12	95.79
Total Pervious	**36.74E+15**	**1,372.1E+12**	**96.27**
Urban Impervious	0.05E+15	0.321E+12	99.35
In-Stream Deposition	0.14E+15	1.12E+12	99.22
TOTAL	**36.9E+15**	**1,373.5E+12**	**96.27**

It is important to note that the reduction percentages in Table 5 reflect the need to reduce the number of fecal coliform bacteria entering the stream and do not imply any specific strategy on how these reductions might be achieved. In order to account for uncertainties that are inherent in any model, the Commonwealth is incorporating staged implementation into the TMDL process (see Section 6).

A TMDL is defined as:

TMDL = WLA + LA + MOS

Where:

WLA = waste load allocation (point sources)

LA = load allocation (non-point sources)

MOS = margin of safety
(USEPA, 1999)

Based on the selected TMDL scenario, the TMDL was calculated as presented in Table 6.

Table 6. Average annual loads for the Christians Creek bacteria TMDL (in col/year)

TMDL	WLA	LA	MOS
1,460E+12	**11.8E+12**	**1,380E+12**	**69.6E+12**

5.2. Consideration of Critical Conditions

EPA regulations at 40 CFR 130.7 (c)(1) require TMDLs to take into account critical conditions for stream flow, loading, and water quality parameters. The intent of this requirement is to ensure that the water quality of Christians Creek is protected during times when it is most vulnerable.

Critical conditions are important because they describe the factors that combine to cause a violation of water quality standards and will help in identifying the actions that may have to be undertaken to meet water quality standards. The sources of bacteria for Christians Creek were a mixture of dry and wet weather driven sources. TMDL development utilized a continuous simulation model that applies to both high and low flow conditions. Therefore, the critical conditions for Christians Creek were addressed during TMDL development.

5.3. Consideration of Seasonal Variations

Seasonal variations involve changes in stream flow and water quality as a result of hydrologic and climatological patterns. Seasonal variations were explicitly included in the modeling approach for this TMDL. The continuous simulation model developed for this TMDL explicitly incorporates the seasonal variations of rainfall, runoff and fecal coliform wash-off by using an hourly time-step. In addition, fecal coliform accumulation rates for each land use were developed on a monthly basis. This allowed the consideration of temporal variability in duck and goose populations within the watershed.

6. TMDL Implementation

The Commonwealth intends for this TMDL to be implemented through best management practices (BMPs) in the watershed. Implementation will occur in stages. The benefits of staged implementation are:

1. as stream monitoring continues to occur, it allows for water quality improvements to be recorded as they are being achieved;
2. it provides a measure of quality control, given the uncertainties which exist in any model;
3. it provides a mechanism for developing public support;
4. it helps to ensure the most cost effective practices are implemented initially; and
5. it allows for the evaluation of the adequacy of the TMDL in achieving the water quality standard.

Watershed stakeholders will have opportunity to participate in the development of the TMDL implementation plan as outlined below. While specific goals for BMP implementation will be established as part of the implementation plan development process, some general guidelines and suggestions are outlined below.

In general, the Commonwealth intends for the required reductions to be implemented in an iterative process that first addresses those sources with the largest impact on water quality. For example, the most promising management practice in agricultural areas of the watershed is livestock exclusion from streams. This has been shown to be very effective in lowering fecal coliform concentrations in streams, both from the cattle deposits themselves and from additional buffering in the riparian zone. Additionally, reducing the human bacteria loading from failing septic systems and leaking sewer lines should be a focus during the first stage because of its health implications. This component could be implemented through education on septic pump-outs as well as a sanitary sewer inspection and management program.

During the second stage of implementation, increased stockpiling of poultry litter may be an appropriate management measure to pursue, following research on the required storage time needed to remove fecal coliform bacteria. Management practices that might be appropriate for controlling urban wash-off from parking lots and roads and that could be readily implemented may include more restrictive ordinances to reduce fecal loads from pets, improved garbage collection and control, and improved street cleaning.

Table 7 shows a progression of potential implementation activities and the resulting improvements in water quality that would be expected based on water quality modeling. Stage 4 is the proposed TMDL scenario, i.e. Scenario 3 in Table 4.

Table 7. Implementation stages for Christians Creek TMDL

Stage #	Percent reduction in loading from existing condition							
	Impervious Land Surface	In-Stream	Pervious Land Surface					Reduction in Geometric Mean
	Dog	Cattle	Human[1]	Pets	Cattle & Poultry	Sheep & Horses	Wildlife	
1	0	90	0	0	30	30	0	62%
2	95	95	100	80	35	35	0	81%
3	95	95	100	80	80	35	0	90%
4	98	99	100	95	95	95	95	97%

[1]Reductions are cumulative off the existing condition geometric mean; to meet water quality standards, a 97% reduction of the average long-term geometric mean fecal coliform concentration in the stream is required. Stage 4 is the proposed TMDL scenario (i.e. scenario 3 in Table 4)
[2]Represents the cumulative effect of failing septic systems, overflowing and leaking sewer lines and illicit connections

7. Reasonable Assurance for Implementation

7.1. Follow-Up Monitoring

The Department of Environmental Quality will continue to monitor Christians Creek in accordance with its ambient monitoring program. VADEQ and VADCR will continue to use data from these monitoring stations to evaluate reductions in fecal bacteria counts and the effectiveness of the TMDL in attaining and maintaining water quality standards.

7.2. Regulatory Framework

This TMDL is the first step toward the expeditious attainment of water quality standards. The second step will be to develop a TMDL implementation plan, and the final step is to implement the TMDL until water quality standards are attained.

Section 303(d) of the Clean Water Act (CWA) and current EPA regulations do not require the development of implementation strategies. However, including implementation plans as a TMDL requirement has been discussed for future federal regulations. Additionally, Virginia’s 1997 Water Quality Monitoring, Information and Restoration Act (WQ MIRA) directs VADEQ in section 62.1-44.19.7 to “develop and implement a plan to achieve fully supporting status for impaired waters”. The Act also establishes that the implementation plan shall include the date of expected achievement of water quality objectives, measurable goals, corrective actions necessary and the associated cost, benefits and environmental impact of addressing the impairments. EPA outlines the minimum elements of an approvable implementation plan in its 1999 “Guidance for Water Quality-Based Decisions: The TMDL Process”. The listed elements include implementation actions/management measures, time line, legal or regulatory controls, time required to attain water quality standards, monitoring plan and milestones for attaining water quality standards. Watershed stakeholders will have opportunities to provide input and to participate in the development of the implementation plan, which will also be supported by regional and local offices of VADEQ, VADCR, and other cooperating agencies.

DEQ acknowledges that it may not be possible to meet the existing water quality standard because of the wildlife issue associated with a number of bacteria TMDLs (see section 7.4 below). At some future time, it may therefore become necessary to investigate the stream’s use designation and adjust the water quality criteria through a Use Attainability Analysis.

Once developed, VADEQ intends to incorporate the TMDL implementation plan into the appropriate Water Quality Management Plan, in accordance with the CWA’s Section 303(e). In response to a Memorandum of Understanding (MOU) between EPA and VADEQ, VADEQ also submitted a draft Continuous Planning Process to EPA in which VADEQ commits to regularly updating the WQMPs. Thus, the WQMPs will be, among other things, the repository for all TMDLs and TMDL implementation plans developed within a river basin.

7.3. Implementation Funding Sources

One potential source of funding for TMDL implementation is Section 319 of the Clean Water Act. In response to the federal Clean Water Action Plan, Virginia developed a Unified Watershed Assessment that identifies watershed priorities. Watershed restoration activities, such as TMDL implementation, within these priority watersheds are eligible for Section 319 funding. Increases in Section 319 funding in future years will be targeted towards TMDL implementation and watershed restoration. Other funding sources for implementation include the USDA's Conservation Reserve Enhancement Program (CREP), the state revolving loan program, and the VA Water Quality Improvement Fund.

7.4. Addressing Wildlife Contributions

In some streams for which TMDLs have been developed, water quality modeling indicates that even after removal of all of the sources of fecal coliform (other than wildlife), the stream will not attain standards. As is the case for Christians Creek, TMDL allocation reductions of this magnitude are not realistic and do not meet EPA's guidance for reasonable assurance. Based on the water quality modeling, many of these streams will not be able to attain standards without some reduction in wildlife. **Virginia and EPA are not proposing the elimination of wildlife to allow for the attainment of water quality standards.** This is obviously an impractical action. While managing over-populations of wildlife remains as an option to local stakeholders, the reduction of wildlife or changing a natural background condition is not the intended goal of a TMDL. In such a case, after demonstrating that the source of fecal contamination is natural and uncontrollable by effluent limitations and BMPs, the state may decide to re-designate the stream's use for secondary contact recreation or to adopt site specific criteria based on natural background levels of fecal coliforms. The state must demonstrate that the source of fecal contamination is natural and uncontrollable by effluent limitations and BMPs through a so-called Use Attainability Analysis (UAA) as described in chapter 3. All site-specific criteria or designated use changes must be adopted as amendments to the water quality standards regulations. Watershed stakeholders and EPA will be able to provide comment during this process.

Based on the above, EPA and Virginia have developed a TMDL strategy to address the wildlife issue. The first step in this strategy is to develop interim reduction goals such as presented in Table 7. The pollutant reductions for the interim goal are applied only to controllable, anthropogenic sources identified in the TMDL, setting aside any control strategies for wildlife. During the first implementation phase, all controllable sources would be reduced to the maximum extent practicable using the staged approach outlined above. Following completion of the first phase, VADEQ would re-assess water quality in the stream to determine if the water quality standard is attained. This effort will also evaluate if the modeling assumptions were correct. If water quality standards are not being met, a UAA may be initiated to reflect the presence of naturally high bacteria levels due to uncontrollable sources. In some cases, the effort may never have to go to the second phase because the water quality standard exceedances attributed to wildlife in the model are very small and infrequent and may fall within the margin of error.

8. Public Participation

The development of the Christians Creek TMDL would not have been possible without public participation The first public meeting was held in the Beam Annex Building next to Expo Hall on Route 935, Fishersville, VA on Thursday, May 24, 2000 to discuss the process for TMDL development. 51 people attended. Copies of the presentation materials were available for public distribution. The meeting was public noticed in the *Virginia Register*. A public meeting notice was also sent to residents of the watershed by DCR staff. There was a 30 day-public comment period and 1 written comment was received. A written response was not required.

The second public meeting was held in the Beam Annex Building next to Expo Hall on Route 935, Fishersville, VA on Wednesday, November 8, 2000 to discuss the source assessment input, bacterial source tracking, and model calibration data. 34 people attended. Copies of the presentation materials were available for public distribution. The meeting was public noticed in the *Virginia Register*. A public meeting notice was also sent to residents of the watershed by DCR staff on October 31, 2000. There was a 30 day-public comment period and 2 written comments were received. A written response was not required.

The third public meeting was held in the Beam Annex Building next to Expo Hall on Route 935, Fishersville, VA on Wednesday, November 2, 2001 to discuss the draft TMDL. 21 people attended. Copies of the draft TMDL were available for public distribution. The meeting was public noticed in the *Virginia Register*. A public notice was sent to residents of the watershed by DCR staff on October 29, 2001, and sent to the following radio stations: WMRA, WSVA, WQPO, WAMM, WHBG, WBHB, WBOP, WKCY on October 29, 2001. There was a 30-day public comment period and 1 written comment was received. A written response was not required.

9. References

USEPA, 1991. Guidance for Water Quality-Based Decisions: The TMDL Process. EPA 440/4-91-001. U.S. Environmental Protection Agency, Washington, DC.

USEPA, 1999. Draft Guidance for Water Quality-Based Decisions: The TMDL Process. EPA 841-D-99-001. U.S. Environmental Protection Agency, Washington, DC.

VADEQ, 1998. Virginia's 1998 303(d) Total Maximum Daily Load Priority List and Report. Virginia Department of Environmental Quality, Richmond, VA.

Appendix A.

Fecal Coliform TMDL Development For Christians Creek, Augusta County, Virginia

USGS Water Resources Investigations Report No. xxxxx

(- Available Upon Request -)

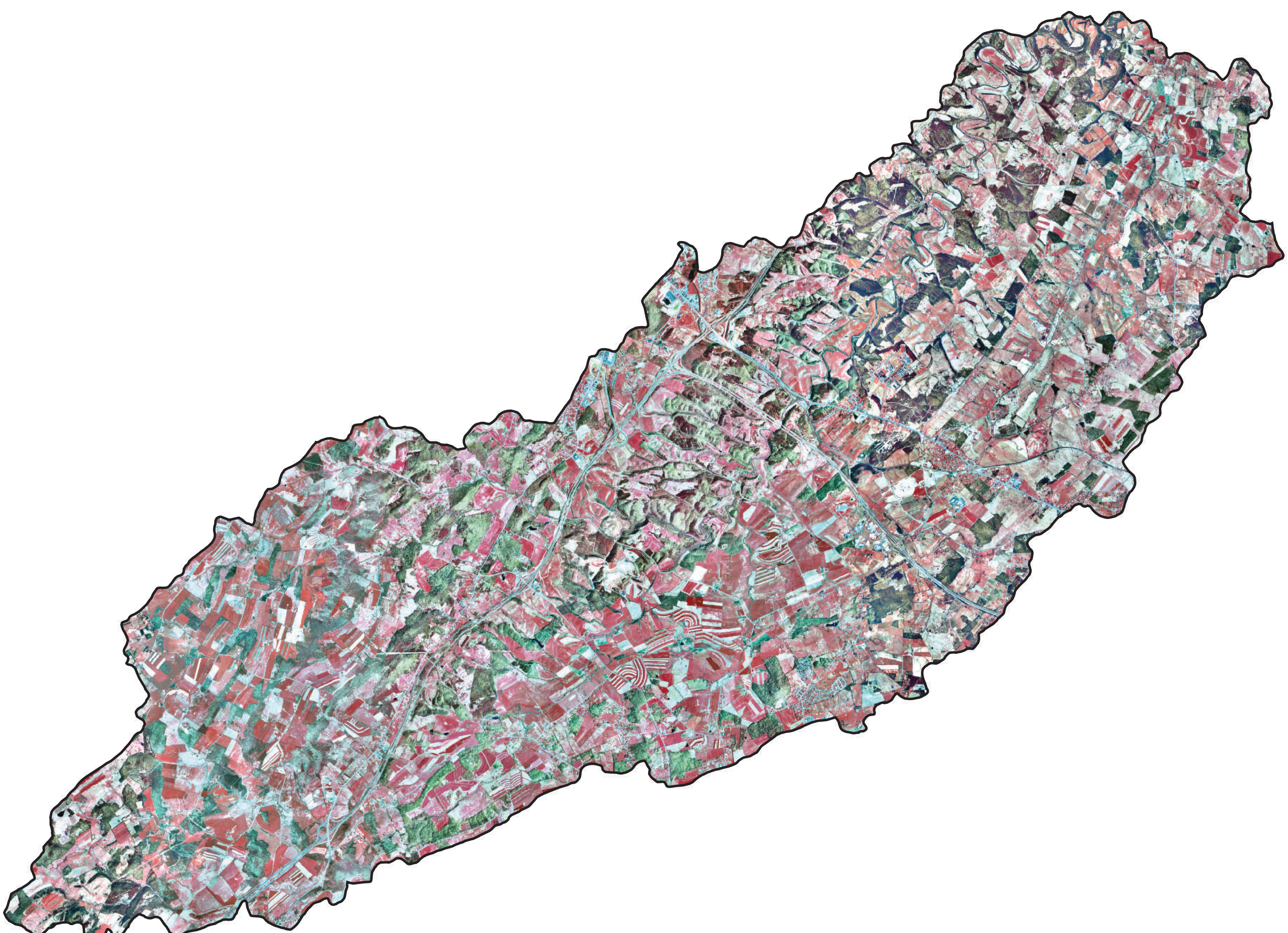
U.S. Department of the Interior
U.S. Geological Survey

Prepared in cooperation with:

Virginia Department of Conservation and Recreation

Use of the Hydrological Simulation Program–FORTRAN and Bacterial Source Tracking for Development of the Fecal Coliform Total Maximum Daily Load (TMDL) for Christians Creek, Augusta County, Virginia

Water-Resources Investigations Report 03-4162



USGS
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Cover image of Christians Creek watershed from U.S. Department of Agriculture-Farm Service Agency-Aerial Photography Field Office, Digital Orthophoto Quadrangle, MrSID mosaic

U.S. Department of the Interior
U.S. Geological Survey

Use of the Hydrological Simulation Program–FORTRAN and Bacterial Source Tracking for Development of the Fecal Coliform Total Maximum Daily Load (TMDL) for Christians Creek, Augusta County, Virginia

By Douglas L. Moyer and Kenneth E. Hyer

Water-Resources Investigations Report 03-4162

Prepared in cooperation with:

Virginia Department of Conservation and Recreation

Richmond, Virginia
2003

U.S. DEPARTMENT OF THE INTERIOR
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CONVERSION FACTORS, DATUM, AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter
foot (ft)	0.3048	meter
mile (mi)	1.609	kilometer
	Area	
acre	4,047	square meter
acre	0.4047	hectare
square mile (mi^2)	259.0	hectare
square mile (mi^2)	2.590	square kilometer
	Volume	
gallon (gal)	3.785	liter
gallon (gal)	0.003785	cubic meter
million gallons (Mgal)	3,785	cubic meter
cubic foot (ft^3)	0.028317	cubic meter
acre-foot (acre-ft)	1,233	cubic meter
	Flow	
cubic foot per second (ft^3/s)	0.02832	cubic meter per second
million gallons per day (Mgal/d)	0.04381	cubic meter per second
inch per hour	0.0254	meter per hour
inch per year	2.54	centimeter per year
	Mass	
ounce, avoirdupois (oz)	28.35	gram (g)
pound, avoirdupois (lb)	0.4536	kilogram
pound per acre (lb/acre)	1.121	kilogram per hectare

Horizontal coordinate information is referenced to the North American Datum of 1927 (NAD27).

Temperature: Temperature is reported in degrees Celsius (°C), which can be converted to degrees Fahrenheit (°F) as follows: °F = 1.8 (°C) + 32°

Abbreviated water-quality units: Bacterial concentrations are reported in units of colonies per 100 milliliters (col/100 mL).

Use of the Hydrological Simulation Program–FORTRAN and Bacterial Source Tracking for Development of the Fecal Coliform Total Maximum Daily Load (TMDL) for Christians Creek, Augusta County, Virginia

By Douglas L. Moyer and Kenneth E. Hyer

ABSTRACT

Impairment of surface waters by fecal coliform bacteria is a water-quality issue of national scope and importance. Section 303(d) of the Clean Water Act requires that each State identify surface waters that do not meet applicable water-quality standards. In Virginia, more than 175 stream segments are on the 1998 Section 303(d) list of impaired waters because of violations of the water-quality standard for fecal coliform bacteria. A total maximum daily load (TMDL) will need to be developed by 2006 for each of these impaired streams and rivers by the Virginia Departments of Environmental Quality and Conservation and Recreation. A TMDL is a quantitative representation of the maximum load of a given water-quality constituent, from all point and nonpoint sources, that a stream can assimilate without violating the designated water-quality standard. Christians Creek, in Augusta County, Virginia, is one of the stream segments listed by the State of Virginia as impaired by fecal coliform bacteria. Watershed modeling and bacterial source tracking were used to develop the technical components of the fecal coliform bacteria TMDL for Christians Creek. The Hydrological Simulation Program–FORTRAN (HSPF) was used to simulate streamflow, fecal coliform concentrations, and source-specific fecal coliform loading in Christians Creek. Ribotyping, a bacterial source tracking technique, was used to identify the dominant sources of fecal coliform bacteria in the Christians Creek watershed. Ribotyping also was used to determine the relative contributions of specific sources to the observed fecal coliform load in Christians Creek. Data from the ribotyping analysis were incorporated into the calibration of the fecal coliform model.

Study results provide information regarding the calibration of the streamflow and fecal coliform bacteria models and also identify the reductions in fecal coliform loads required to meet the TMDL for Christians Creek. The calibrated streamflow model simulated observed streamflow characteristics with respect to total annual runoff, seasonal runoff, average daily streamflow, and hourly stormflow. The calibrated fecal coliform model simulated the patterns and range of observed fecal coliform bacteria concentrations. Observed fecal coliform bacteria concentrations during low-flow periods ranged from 40 to 2,000 colonies per 100 milliliters, and peak concentrations during stormflow periods ranged from 23,000 to 730,000 colonies per 100 milliliters. Additionally, fecal coliform bacteria concentrations were generally higher upstream and lower downstream. Simulated source-specific contributions of fecal coliform bacteria to instream load were matched to the observed contributions from the dominant sources, which were beaver, cats, cattle, deer, dogs, ducks, geese, horses, humans, muskrats, poultry, raccoons, and sheep. According to model results, a 96-percent reduction in the current fecal coliform load delivered from the watershed to Christians Creek would result in compliance with the designated water-quality goals and associated TMDL.

INTRODUCTION

Background

Surface-water impairment by fecal coliform bacteria is a water-quality issue of national scope and importance. Section 303(d) of the Clean Water Act requires that each State identify surface waters that do not meet applicable water-quality standards. In Virginia, more than 175 stream segments are on the 1998 Section 303(d) list of impaired waters because of violations of the fecal coliform bacteria standard (an instantaneous water-quality standard of 1,000 col/100 mL, or a geometric mean water-quality standard of 200 col/100 mL). Christians Creek, in Augusta County, Virginia (fig. 1), is one of these impaired streams. Fecal coliform bacteria concentrations that are elevated above the State water-quality standard indicate an increased risk to human health when these waters are contacted through swimming or other recreational activities.

In Virginia, total maximum daily load (TMDL) plans will need to be developed by 2006 for impaired waterbodies on the State 1998 Section 303(d) list. TMDLs are a quantitative representation of all the contaminant contributions to a stream and are defined as

$$TMDL = \Sigma WLAs + \Sigma LAs + MOS \quad (1)$$

where $\sum$ WLAs (waste-load allocations) represents the sum of all the point-source loadings, $\sum$ LAs (load allocations) represents the sum of all the nonpoint-source loadings, and MOS represents a margin of safety. The sum of these loading terms and assigned margin of safety constitute the TMDL and represent the loading of a particular constituent that the surface waterbody can assimilate without violating the State water-quality standard. The TMDL must meet eight conditions in order to be approved by the U.S. Environmental Protection Agency (USEPA). These conditions ensure that the TMDL (1) is designed to implement applicable water-quality standards; (2) includes a total allowable load as well as individual waste-load allocations and load allocations; (3) considers the effect of background contaminant contributions; (4) considers critical environmental conditions (periods when water quality is most affected); (5) considers seasonal variations; (6) includes a margin of safety; (7) has been subject to public participation; and (8) can be met with reasonable assurance. Once a TMDL is established, source-load contributions then can be reduced through implementation of source-control management practices until the target TMDL is achieved.

In Virginia, the primary tool for developing TMDLs in impaired watersheds has been the Hydrological Simulation Program–FORTRAN (HSPF) watershed model. HSPF is a continuous simulation watershed model designed to simulate the transport and storage of water and associated water-quality constituents by linking surface, soil, and instream processes (Donigian and others, 1995). HSPF recently has been demonstrated to be an effective tool for the simulation of fecal coliform bacteria for TMDL development (U.S. Environmental Protection Agency, 2000). HSPF has been used extensively to simulate watershed hydrology (Ng and Marsalek, 1989; Donigian and others, 1995; Berris, 1996; Dinicola, 1997; Srinivasan and others, 1998; Zarriello, 1999) and water-quality constituents such as nutrients in agricultural runoff (Bicknell and others, 1985; Donigian, 1986; Moore and others, 1988; Linker and others, 1996), sediment (Sams and Witt, 1995; Fontaine and Jacomino, 1997), atrazine (Laroche and others, 1996), and water temperature (Chen and others, 1998).

One of the major difficulties in developing TMDLs for waters contaminated by fecal coliform bacteria is that the potential sources of bacteria are numerous and the magnitude of their contributions commonly is unknown. Potential sources of fecal coliform bacteria include all warm-blooded animals (humans, pets, domesticated livestock, birds, and wildlife). The lack of information on the bacteria sources hinders the development of accurate load allocations and the identification of appropriate source-load reduction measures. Information about the major fecal coliform sources that impair surface-water quality would improve the ability to develop effective watershed models and may lead to more scientifically defensible TMDLs.

Bacterial source tracking (BST) is a recently developed tool for identifying the sources of fecal coliform bacteria found in surface waters (Hyer and Moyer, 2003). This technology identifies specific differences among fecal coliform bacteria present in the feces of different animal species. Time, diet, environment, and many other factors may have contributed to produce these evolutionary distinctions; BST uses these species-specific distinctions to identify the animal source

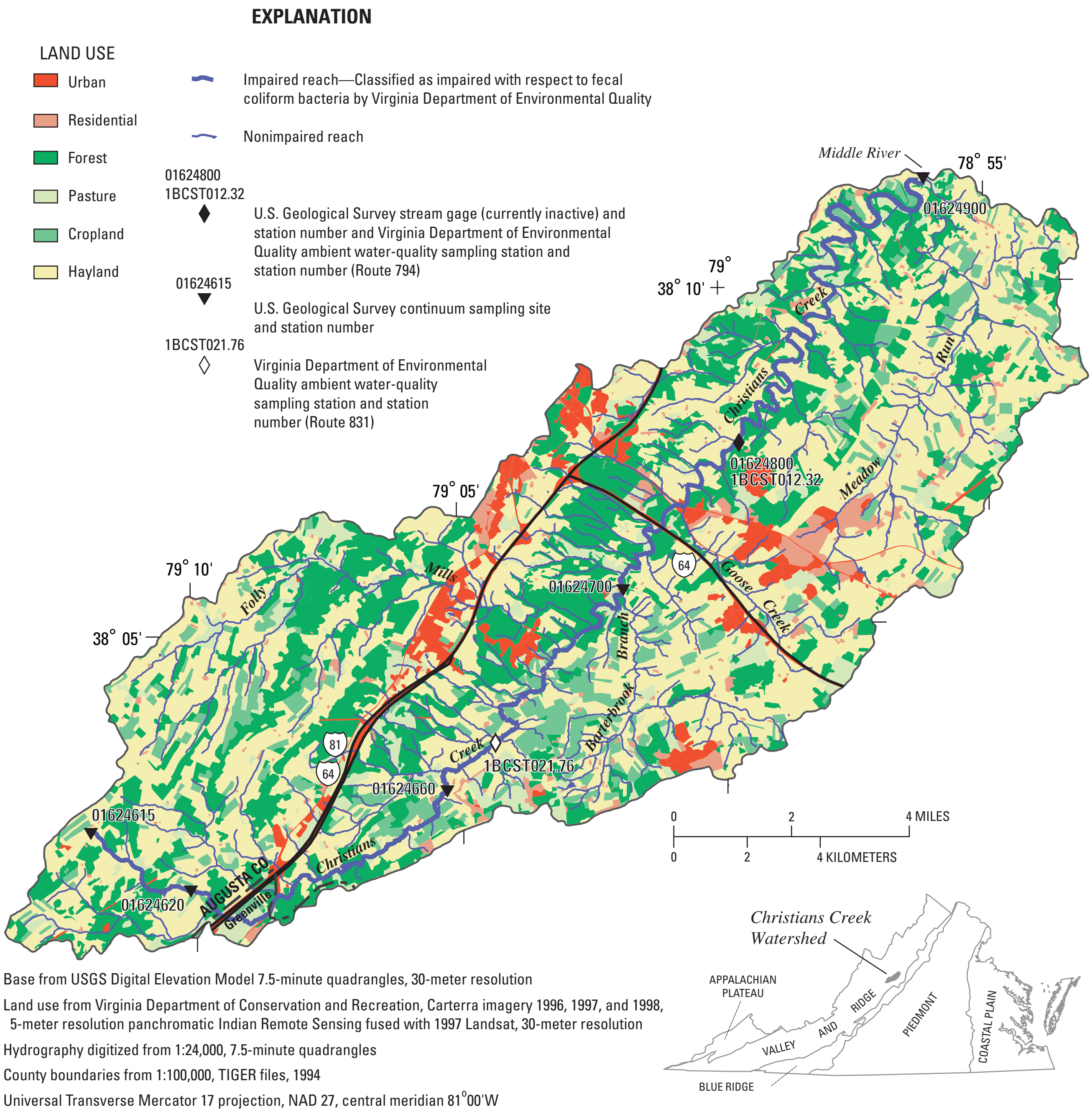


Figure 1. Land use, streams, stream-gaging station, and water-quality sampling stations in the Christians Creek watershed, Augusta County, Virginia.

of an unknown fecal coliform that has been isolated from a waterbody. The BST method chosen to identify the dominant sources of fecal coliform bacteria in the Christians Creek watershed is ribotyping (Hyer and Moyer, 2003), which involves an analysis of the specific DNA (deoxyribonucleic acid) sequence that codes for the production of ribosomal RNA (ribonucleic acid). Ribotyping identifies bacteria sources with a degree of precision that makes it well suited for use in the development of a fecal coliform TMDL.

In 1999, the U.S. Geological Survey (USGS), in cooperation with the Virginia Department of Conservation and Recreation (DCR), began a 3-year study to develop a fecal coliform bacteria TMDL for the Christians Creek watershed. The primary objective was to develop a HSPF model to simulate streamflow and the transport of fecal coliform bacteria within the watershed. Specific project objectives were to (1) produce calibrated models of watershed streamflow and fecal coliform bacteria transport, (2) incorporate BST information into the fecal coliform model calibration process, (3) estimate fecal coliform source-load reductions required to meet State water-quality standards, and (4) define the TMDL for fecal coliform bacteria for Christians Creek. These objectives ensure that the Christians Creek TMDL would (1) include a total allowable load as well as individual waste load and load allocations; (2) consider the effect of background contaminant contributions; (3) consider critical environmental conditions; (4) consider seasonal variations; and (5) include a margin of safety. The primary objectives for DCR were to ensure that the Christians Creek TMDL was designed to implement applicable water-quality standards; was developed with public participation; and can be met with reasonable assurance.

Purpose and Scope

This report describes the development and calibration of the HSPF model for streamflow and fecal coliform bacteria as part of determining the TMDL for the Christians Creek watershed. The model simulation period is from October 1991 to September 1997. This report also documents the methodology for incorporating BST data into the calibration of the fecal coliform model and demonstrates how these data enhance TMDL development. Current source-specific fecal coliform bacteria loads in Christians Creek are presented as well as the load reductions needed to meet the designated TMDL and associated State water-quality standard.

Christians Creek Watershed Characteristics

Christians Creek, located in Augusta County, Va., originates northwest of Greenville, Va., and extends to the confluence with the Middle River. The entire 31.5-mi-long reach is classified as impaired with respect to fecal coliform bacteria (Virginia Department of Environmental Quality, 1998). The basin has a drainage area of 107 mi^2, and an estimated population of 12,000 (1990 Census). A recently deactivated USGS stream gage (station number 01624800; fig. 1), still operational for instantaneous stage determinations, is at Route 794 (Sangers Lane), and has a period of record from 1967 to 1997. DEQ has performed monthly sampling of fecal coliform bacteria at Route 794 and also at Route 831 (Old White Hill Road) (DEQ station number 1BCST021.76; fig. 1) since 1991. Route 794 was the primary sampling site for this study.

The Christians Creek watershed lies in the Valley and Ridge physiographic province. Underlying the watershed are 10 geologic formations dominated by limestone and dolomite; information about each formation is summarized from the work of Rader (1967). The Martinsburg Formation (calcareous shale and sandstone) is the dominant formation within the basin. Other formations in the watershed include the Edinburg Formation (argillaceous limestone and shale), Lincolnshire Formation (cherty limestone), New Market Limestone (limestone with dolomite beds near the base), Beekmantown Formation (dolomite and limestone), Chepultepec Formation (limestone and dolomite), Conococheague Formation (limestone, dolomite, and sandstone), and Elbrook Formation (limestone and dolomite). Karst features, such as sinkholes and caves, are evident in portions of the watershed. Alluvial material (composed of sand and clay) is present in portions of the floodplain adjacent to Christians Creek. Small amounts of fault breccia (large blocks of dolomite and limestone with crush conglomerate) also occur in the basin.

The soils of the Christians Creek watershed have been described thoroughly (Hockman and others, 1979) and are best classified as a product of their parent material. Much of the soil in the watershed has formed from the residuum of interbedded limestone, dolomite, and calcareous shale. Three soil assemblages have been identified in this category. The Frederick-Christian-Rock outcrop assemblage consists of deep, well-drained, silt loam or fine sandy loam soils with limestone outcrop areas. The Frederick-Bookwood-Christian assemblage consists of deep to moderately deep, well-drained, silt loam or fine sandy loam soils; scattered sinkholes or rock outcrops also may occur. The Chilhowie-Edom assemblage consists of deep to moderately deep, well drained, silt loam or silty clay loam soils with occasional bedrock outcrops. Considerable soil has also formed from the residuum of shale and thin interbedded sandstone and limestone. These soils are part of the Berks-Weikert-Sequoia assemblage, which consists of shallow to deep, well-drained, silt loam or shaly silt loam soils. On flood plains and terraces, soils have formed in the alluvial or colluvial material. Although not extensive within the watershed, these soils are part of the Buchanan-Wheeling-Buckton assemblage, which consists of deep, somewhat poorly drained to well-drained soils. These are generally silt loam, loam, or fine sandy loam soils, although some areas are gravelly or cobbly.

Land use in the watershed is dominated by agricultural practices that are potential sources of fecal coliform bacteria in the watershed. Major components of the animal husbandry in this watershed include the production of beef cattle, dairy cattle, heifers, broilers, and turkeys. Other potential sources of fecal coliform bacteria within the watershed are human-related (failing septic systems, leaking or overflowing sewer lines, cross-pipes, and straight pipes), domestic animals (dogs and cats), waterfowl (geese, ducks, and sea gulls), and other wildlife (such as deer, raccoons, opossum, rabbits, muskrats, ground hogs, foxes, and beaver).

Modeling Approach

Streamflow and bacterial transport in the Christians Creek watershed were simulated by means of the Hydrological Simulation Program–FORTRAN (HSPF) version 11 (Bicknell and others, 1997). HSPF is a continuous simulation and lumped parameter watershed model that is used to simulate the transport and storage of water and associated water-quality constituents by linking surface, soil, and instream processes (Donigian and others, 1995). HSPF represents these mechanisms of transport and storage for three unique land segments or model elements: pervious land segments (PERLND), impervious land segments (IMPLND), and stream channels (RCHRES). Natural variability in these hydrologic transport mechanisms occurs because of spatial changes in watershed characteristics such as topography, land use, and soil properties; HSPF accounts for this variability by simulating runoff from smaller, more homogeneous portions of the watershed. Thus, for modeling purposes, the watershed is disaggregated into subwatersheds with similar land-use and topographical features. Each subwatershed is refined further into hydrologic response units (HRU) that represent areas within each land segment with similar watershed characteristics such as land use (Leavesley and others, 1983). HSPF links the movement of water and constituents from each HRU to generate an overall watershed response.

Acknowledgments

We thank DCR for their cooperation, input, and assistance with this study. The Virginia Department of Environmental Quality (DEQ) is acknowledged for their technical support and assistance. The authors also would like to thank Tom Jobes (Aqua Terra Consultants) for his help with the development of the model. Michael J. Gearheart, Trisha L. Baldwin, and Robert R. Lotspeich provided outstanding technical support on this project. Allison A Shipp and Michael E. Lewis are acknowledged for providing technical reviews of this report. Lastly, we are indebted to the residents of and stakeholders in the Christians Creek watershed for their enthusiastic participation in this study. Without their cooperation, this study would not have been possible.

DESCRIPTION OF MODELS

The following sections describe the streamflow and fecal coliform bacteria models used in this study for development of the fecal coliform TMDL for the Christians Creek watershed.

Streamflow Model

The first step in generating a watershed-scale bacterial transport model is the simulation of streamflow. The mechanisms by which precipitation is routed from the land surface, through the various soil layers, and to the stream channel must be represented accurately in order to build a bacterial transport model. The following sections summarize the transport mechanisms associated with the PERLND, IMPLND, and RCHRES modules. A detailed description of the hydrologic portion of HSPF is in Bicknell and others (1997).

Pervious and Impervious Land Segments

The dominant feature of the pervious land segment (PERLND) module is the component for calculating the hydrologic water budget (PWATER). PWATER includes parameters that represent storage (vegetative, surface, shallow subsurface, and deep subsurface) and transport (evaporation, transpiration, inflow, and outflow) components of the hydrologic cycle (table 1). PWATER simulates the storage and transport of precipitation along three flow paths: overland flow, interflow (shallow subsurface flow), and base flow (active ground-water discharge). Storage and transport parameters are refined to simulate the hydrologic routing through each HRU, generating a simulated watershed response between and during precipitation events.

The simulated hydrologic cycle indicates how these storage and transport parameters govern the overall stream response within the watershed (fig. 2). Precipitation falling on the watershed is first intercepted (CEPSC) and stored by the vegetation. Most of the precipitation then is routed to the land surface because the surface area of the intercepting vegetation is small relative to the total volume of precipitation. The volume of water that remains on the vegetation is lost to the atmosphere through evaporation.

Water that falls on the land surface is captured and stored temporarily (SURS) before being transported along three potential pathways: (1) Stored water begins to infiltrate the subsurface (INFILT). The infiltrating water is distributed among the upper-zone storage (UZSN), lower-zone storage (LZSN), active ground-water storage (AGWS), and inactive ground-water storage. (2) Water also is routed to interflow storage (IFWS) just beneath the land surface. This pathway is active when the deeper subsurface storages are full and the rate of precipitation approaches the rate of infiltration. Water held in interflow storage is released as interflow to the stream. The residence time for the stored water is governed by the interflow recession constant (IRC). (3) The stored water is routed directly to the stream through overland flow. This pathway is active when all subsurface storages are full and/or the precipitation rate exceeds the infiltration capacity of the soils. Overland flow is governed by the length (LSUR), slope (SLSUR), and roughness (NSUR) of the overland flow path.

Water in upper-zone storage (UZSN) ultimately is lost to the atmosphere (through evapotranspiration) and the deeper subsurface (through delayed infiltration). Water that infiltrates to the deeper subsurface will be divided among lower-zone storage (LZSN), inactive ground-water storage, and active ground-water storage (AGWS). Water stored in the lower zone can be lost to the atmosphere through evapotranspiration (LZETP). Water that is transported to inactive ground-water storage is lost from the simulated basin and is never transported to the simulated stream reach. The portion of infiltrating water that is allocated to inactive ground-water storage is governed by DEEPFR. Water that enters AGWS either through delayed infiltration from UZSN or through direct infiltration from surface storage is either lost to the atmosphere through evapotranspiration (AGWETP) or transported to the simulated stream reach through base flow. The residence time for water in AGWS storage is controlled by AGWETP and the active ground-water recession constant (AGWRC). Finally, a portion of the base flow is removed through evapotranspiration (BASETP) prior to entering the stream channel.

The component under the impervious land segment (IMPLND) module that calculates the hydrologic water budget is IWATER. Simulation of the flux and storage of precipitation falling on impervious land segments is less complex than for pervious land segments because there are no infiltration and subsurface processes. Similar to PWATER, IWATER contains parameters that represent the storage (rooftop and surface) and transport (evaporation and runoff) components of the hydrologic cycle. These parameters are unique to each impervious HRU so that precipitation runoff may be simulated accurately.

The routing of precipitation in IWATER is similar to the surface runoff routing in the PERLND module. Precipitation that falls on the watershed is first intercepted by impervious surfaces (building tops, urban vegetation, and asphalt wetting) that extend above the land surface (impervious retention storage – RETS). Most of the precipitation is passed to the land surface because the storage capacity of the intercepting surfaces is relatively small compared to the volume of incoming precipitation. The water that remains in

Table 1. Hydrologic parameters used in the simulation of streamflow in Christians Creek, Augusta County, Virginia

[ET, evapotranspiration; PET, potential evapotranspiration]

Parameter	Definition	Unit
AGWETP	Active ground-water ET. Represents the fraction of stored ground water that is subject to direct evaporation and transpiration by plants whose roots extend below the active ground-water table. Accounts for the fraction of available PET that can be met from active ground-water storage.	none
AGWRC	Active ground-water recession rate. Represents the ratio of current ground-water discharge to that from 24 hours earlier.	1 per day
BASETP	Base flow ET. ET by riparian vegetation from active ground water entering the stream channel. Represents the fraction of PET that is fulfilled only as ground-water discharge is present.	none
CEPSC	Interception storage capacity of vegetation.	inches
DEEPFR	Fraction of infiltrating water that is lost to deep aquifers. Represents the fraction of ground water that becomes inactive ground water and does not discharge to the modeled stream channel.	none
INFEXP	Infiltration equation exponent.	none
INFILD	Ratio of maximum and mean soil-infiltration capacities.	none
INFILT	Index to mean soil infiltration rate. INFILT governs the overall division of available moisture between surface and subsurface flow paths. High values of INFILT divert more water to the subsurface flow paths.	inches per hour
INTFW	Interflow coefficient that governs the amount of water that enters the ground from surface detention storage.	none
IRC	Interflow retention coefficient. Rate at which interflow is discharged from the upper-zone storage.	1 per day
KVARY	Ground-water recession flow parameter. Describes nonlinear ground-water recession rate.	1 per inch
LSUR	Length of the overland flow plane.	feet
LZETP	Lower-zone evapotranspiration ET. Percentage of moisture in lower-zone storage that is subject to ET.	none
LZSN	Lower-zone nominal storage. Defines the storage capacity of the lower-unsaturated zone.	inches
NSUR	Surface roughness (Manning's n) of the overland flow plane.	none
RETS	Retention-storage capacity of impervious surfaces.	inches
SLSUR	Average slope of the overland flow path.	none
UZSN	Upper-zone normal storage. Defines the storage capacity of the upper-unsaturated zone.	inches

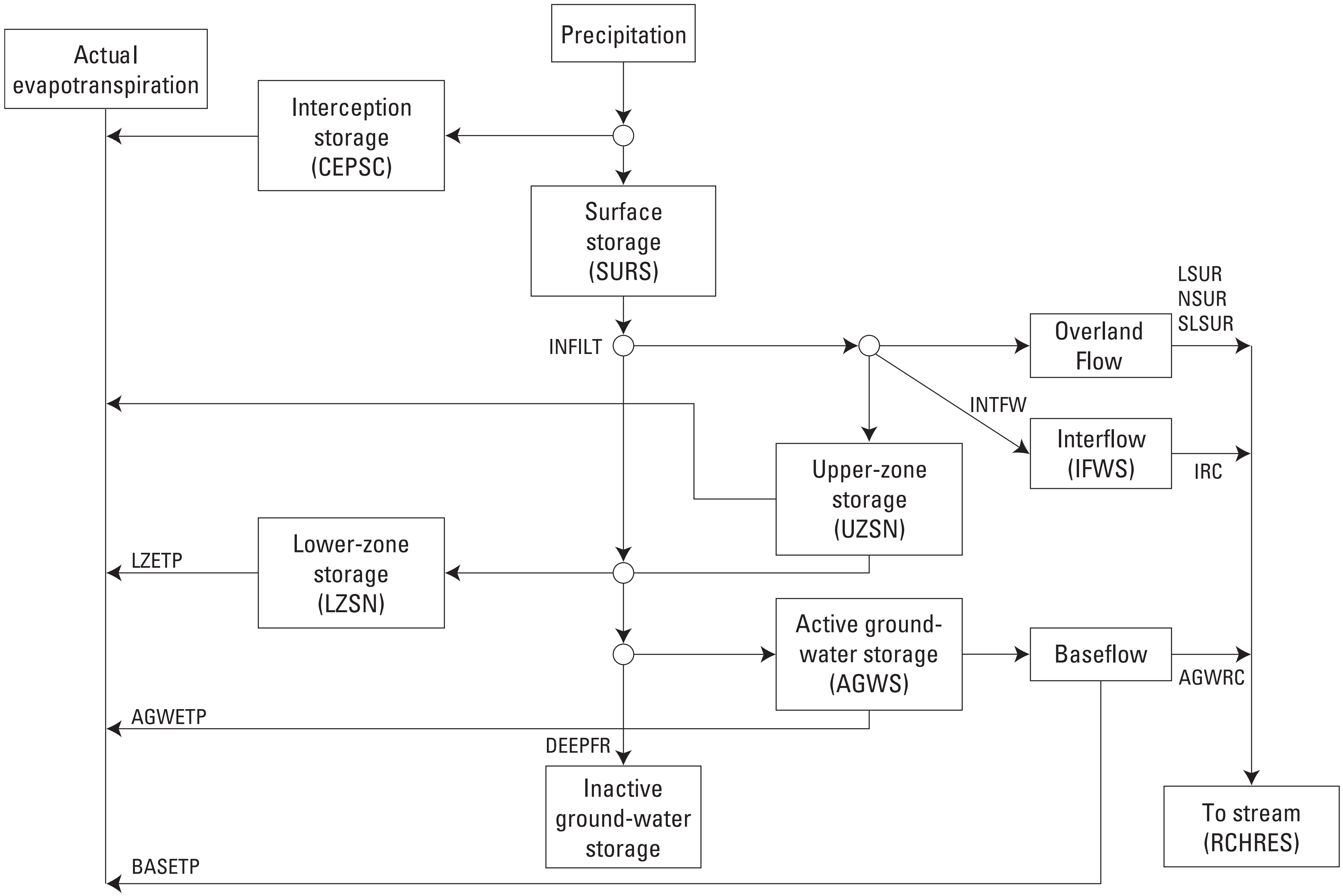


Figure 2. Rainfall-routing processes, associated with pervious land segments, represented by the Hydrological Simulation Program-FORTRAN for the simulation of streamflow in Christians Creek, Augusta County, Virginia. (See table 1 for definition of hydrologic parameters.)

RETS is lost to the atmosphere through evaporation. Water that is routed to the land surface is captured and momentarily stored in surface-detention storage (SURS). This stored water then is transported to the simulated stream reach as surface runoff. Overland flow is governed by the length (LSUR), slope (SLSUR), and roughness (NSUR) of the overland flow path.

The urban and pasture (primarily loafing lots or heavily grazed areas adjacent to the stream channel) land segments represented in the model contain both pervious and impervious features. The main objective associated with the calibration of the impervious area represented in the model is to determine the fraction of impervious area within the urban and pasture land types. This impervious fraction can be broken into two categories, “hydrologically effective” or “hydrologically ineffective” (Zarriello, 1999). Hydrologically effective areas drain directly to stream channels and are represented by the IMPLND module. Hydrologically ineffective areas drain onto pervious land types, such as grassland or forest, and are better represented by the PERLND module. For example, rain that falls on a rooftop, and then is transported to a grassy lawn, would be considered hydrologically ineffective. Initial estimates were that urban land use contains between 18- and 50-percent and pasture contains 1-percent effective impervious (Northern Virginia Planning District Commission, 1980). This initial estimate was refined during model calibration of stormflow timing and magnitude. For instance, overestimating the impervious area will cause a greater volume of water to be routed directly to the stream through surface runoff (in contrast to the delayed response associated with pervious land segments) during a storm event; thus, the simulated storm response will be earlier and of greater magnitude than the observed storm response.

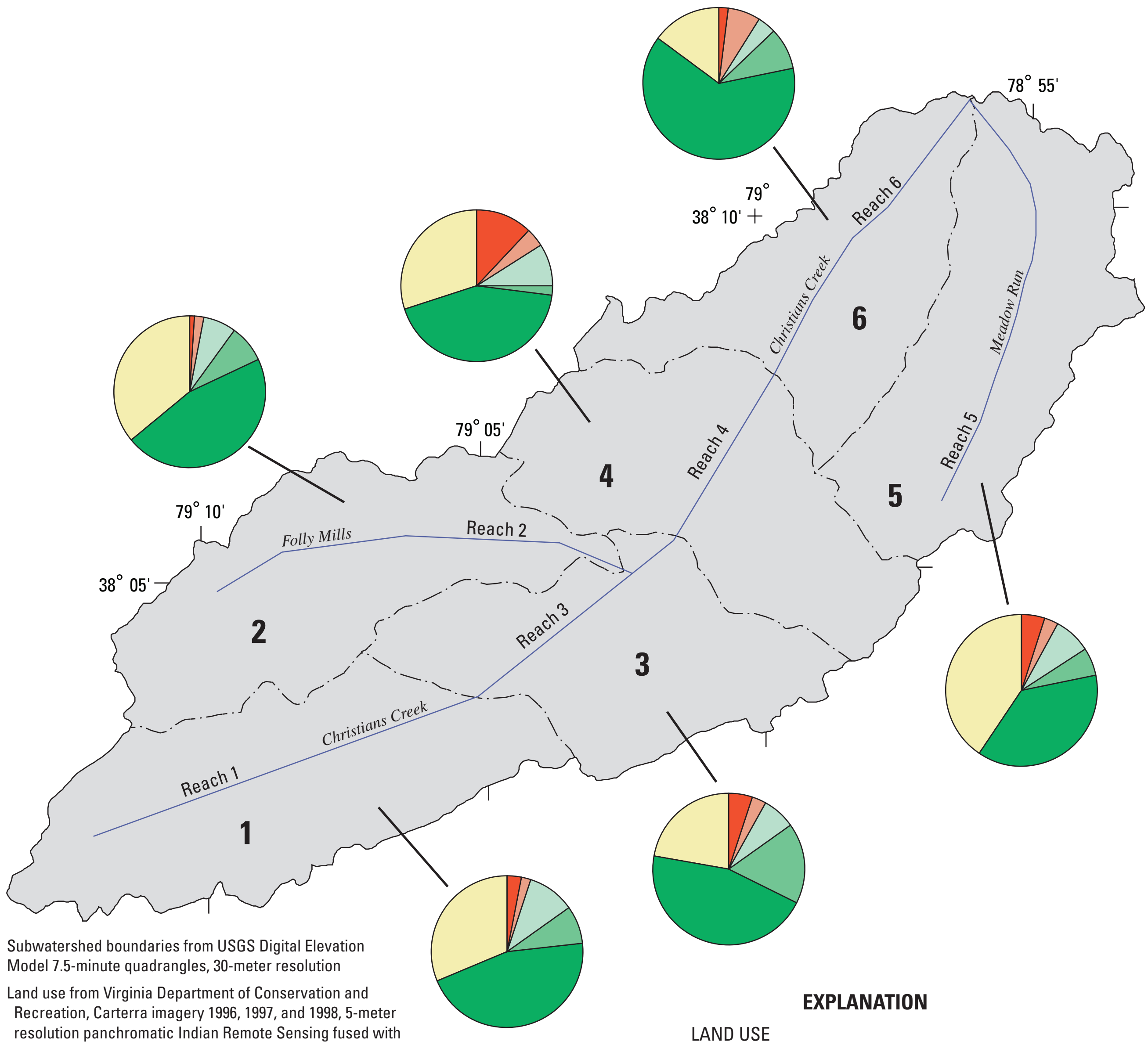
Stream Channels

The RCHRES module in HSPF is used to simulate the routing of water and associated water-quality constituents through a stream channel network that consists of a series of connected stream reaches. For this study, only one reach was simulated within each subwatershed. Water is supplied to a reach from PERLND (overland flow, interflow, and base flow), IMPLND (overland flow), point sources (sewage-treatment plants or STPs), and upstream segments. These inflows are assumed to enter the reach at a single upstream point and the water is transported downstream in a unidirectional manner. Actual channel properties (width, depth, cross-sectional area, slope, and roughness) are measured in order to develop the relation among stage (water depth), surface area, volume, and discharge (streamflow). Stage, surface area, volume, and discharge information are specified in a function table (FTABLE) and are used to govern stream discharge for a given inflow. Water transported down a reach is assumed to follow the kinematic wave function (Martin and McCutcheon, 1999).

Subwatershed Delineation

A critical step in the simulation of streamflow and bacterial transport within a watershed is characterization of the watershed morphology. The morphology consists of watershed characteristics such as topography (slope, aspect, and elevation), soil types, and land use. Within the watershed boundary, each of these characteristics typically is highly variable. For example, the southern portion of the Christians Creek watershed has a higher elevation and steeper slopes than the northern portion. To account for these topographical variations within HSPF, the watershed is broken into smaller, more homogeneous subwatersheds. There also may be variations in land use within each subwatershed; land uses with similar hydrologic responses are grouped into a single HRU. For example, high-intensity residential and high-intensity commercial are assumed to have similar hydrologic responses and were grouped to form an urban HRU. The following section documents the methods used to delineate subwatersheds, aggregate land uses, and establish the stream channel network for the Christians Creek watershed.

Six subwatersheds were identified within the Christians Creek watershed on the basis of variations in land-surface elevation and slope (fig. 3). The area of each subwatershed was determined by delineating along the natural drainage boundary. These drainage boundaries were identified using the USGS Digital Elevation Model (DEM) from the Greenville, Stuarts Draft, Staunton, Waynesboro West, and Fort Defiance 7.5-minute quadrangles. The DEM coverage has a cell size of 30 meters.



Sub-watershed	Land use, in percent					
	Urban	Residential	Pasture	Cropland	Hayland	Forest
1	3	2	10	8	45	31
2	1	2	7	8	46	36
3	5	3	7	17	45	22
4	12	4	9	2	43	30
5	5	3	8	6	38	41
6	2	7	4	9	64	15

Totals may not add to 100 percent because of rounding or other categories not listed.

Figure 3. Hydrologic subwatersheds, land use, and reaches as represented in the streamflow and fecal coliform models for Christians Creek, Augusta County, Virginia.

Land Use

DCR provided land-use data in the form of a Geographic Information System (GIS) coverage for the Christians Creek Watershed. The GIS coverage provides land-use/land-cover information. The land-use coverage identifies 19 possible land-use types, which were combined into 6 general types based on hydrological routing similarities: urban, residential, cropland, hayland, pasture, and forest (table 2). Each of these general land-use types represents the HRUs for each subwatershed.

Table 2. Aggregated hydrologic response units used to develop the watershed model for Christians Creek, Augusta County, Virginia

[Land-use data from Virginia Department of Conservation and Recreation]

Hydrologic Response Unit	Area	
	Acres	Percent of watershed
Urban[1]	3,208	4.7
Residential[2]	2,357	3.4
Cropland	5,379	8.3
Hayland[3]	32,523	47.3
Pasture[4]	5,066	7.4
Forested[5]	19,896	28.9

[1] Includes urban impervious, medium-density residential, high-density residential, commercial and services, industrial, transportation, mixed urban or built up, open urban land, and barren.

[2] Includes residential impervious, low-density residential, mobile home park, wooded residential, poultry operations, and farmstead.

[3] Includes improved pasture and permanent hay.

[4] Includes pasture impervious, unimproved pasture and grazed woodland.

[5] Includes harvested forest land.

Channel Network

A single stream channel (reach) is represented in each of the six subwatersheds simulated in HSPF. The routing of runoff from one reach to a connected downstream reach is governed by the stage, cross-sectional area, storage, and discharge information contained in the FTABLE. An FTABLE was created for each stream reach by first collecting data on stream channel morphology. Stream-channel surveys (transects) were performed by USGS at both the upstream and downstream ends of each reach based on techniques described in Davidian (1984). At each transect, coordinate data (depth at a given position along the transect) were recorded. Estimates of channel roughness (Manning's n) were made on the basis of channel median grain size, irregularity (width to depth ratios), alignment (abrupt changes in channel width), obstructions (debris), vegetation (instream and bank vegetation), and meandering (Barnes, 1967; Arcement and Schneider, 1989; Coon, 1998). Channel slope was estimated by dividing the change in elevation from the upstream and downstream transects by the reach length. Transect coordinate data were loaded into the Channel Geometry Analysis Program (CGAP) to identify the area, width, wetted perimeter, and hydraulic radius of cross sections at successive water-surface elevations (Regan and Schaffranek, 1985). These data from CGAP along with channel roughness and channel slope were loaded into the program Generate FTABLE (GENFTBL, provided with CGAP). GENFTBL creates an FTABLE for each stream reach as required by HSPF. The stage and discharge information (rating table) from the stream gage at Route 794 (USGS station 01624800) was incorporated into the FTABLE for reach segment 4.

Six subwatersheds (1–6) represent the morphological features of the Christians Creek watershed (fig. 3). Within each subwatershed there are 8 HRUs, including 6 pervious (urban, residential, cropland, hayland, pasture, and forest) and 2 impervious areas (urban and pasture). Each subwatershed has a single reach that is governed by an FTABLE. Reaches 1, 3, 4, and 6 represent Christians Creek. Reaches 2 and 5 represent the Folly Mills and Meadow Run tributaries, respectively.

Meteorological and Streamflow Data

Rainfall data were obtained from the National Climatic Data Center. These data are collected hourly at the Staunton Sewage Treatment Plant (SSTP) rain gage that is approximately 6 mi west of the USGS stream gage on Christians Creek. This rain gage has been operational since August 1, 1948. Average annual rainfall measured between 1991 and 1997 was 40.2 in., with a maximum annual rainfall amount of 52.0 in. in 1996 and a minimum annual rainfall amount of 35.1 in. in 1991. The 30-year average rainfall at the SSTP gage is 41.1 in. (Climatological Data Annual Summary for Virginia, 1999). Missing data in the hourly rainfall record were supplemented with data from the She-

Table 3. Meteorological and streamflow data used in the streamflow model for Christians Creek, Augusta County, Virginia

[in., inches; °F, degrees Fahrenheit; NCDC, National Climatic Data Center; NWS, National Weather Service; ft^3/sec, cubic feet per second]

Type of data	Location of data collection	Latitude Longitude	Source	Recording frequency	Period of record
Rainfall (in.)	Staunton Sewage Treatment Plant	38°10′52″ 79°05′25″	NCDC	hourly daily	1/1/73–12/31/99 8/1/48–12/31/99
Rainfall (in.)	Sherando	37°59′45″ 78°59′30″	NWS	hourly	4/1/91–12/31/99
Rainfall (in.)	Spottswood	37°57′42″ 79°12′44″	NWS	hourly	4/1/91–12/31/99
Rainfall (in.)	Middlebrook	38°02′54″ 79°13′45″	NWS	hourly	4/1/91– 12/31/99
Rainfall (in.)	Stoney Creek	37°59′24″ 79°07′22″	NWS	hourly	10/1/93–12/31/99
Minimum air temperature (°F)	Staunton Sewage Treatment Plant	38°10′52″ 79°05′25″	NCDC	daily	8/1/48–12/31/99
Maximum air temperature (°F)	Staunton Sewage Treatment Plant	38°10′52″ 79°05′25″	NCDC	daily	8/1/48 – 12/31/99
Minimum air temperature (°F)	Dale Enterprise Weather Station	38°27′19″ 78°56′07″	NCDC	daily	8/1/48– 2/31/99
Maximum air temperature (°F)	Dale Enterprise Weather Station	38°27′19″ 78°56′07″	NCDC	daily	8/1/48– 2/31/99
Cloud cover (percent)	Lynchburg Regional Airport	37°20′15″ 79°12′24″	NCDC	hourly	8/1/48–6/30/96
Cloud cover (percent)	Quantico Marine Corp Air Station (MCAS)	38°30′00″ 77°18′00″	NCDC	hourly	4/1/45–5/31/98
Dew point temperature (°F)	Lynchburg Regional Airport	37°20′15″ 79°12′24″	NCDC	hourly	1/1/48–6/30/96
Wind speed (360° and knots)	Elkins-Randolph Airport, Elkins, W.Va.	38°53′07″ 79°51′10″	NCDC	hourly	1/1/64–12/31/99
Streamflow (ft^3/sec)	Christians Creek at Fishersville (Route 794)	38°07′42″ 78°59′41″	USGS	hourly daily	10/1/90–9/30/97 10/1/67–9/30/97

rando, Spottswood, Middlebrook, and Stoney Creek rain gages in and around the Christians Creek watershed (table 3). These gages are part of the National Weather Service's Automated Flood Warning System for Augusta County, Va. Data gaps were filled primarily with data from the Middlebrook rain gage, which is nearest to the SSTP. For the 1991–94 time period, data from Spottswood were used when rainfall data from both SSTP and Middlebrook were missing. For 1995–97, average rainfall data from Spottswood, Sherando, and Stoney Creek (activated in 1995) rain gages generally were used when data from both SSTP and Middlebrook were missing.

Daily minimum temperature, daily maximum temperature, percent cloud cover, dew-point temperature, and wind-speed data were collected for the purpose of calculating potential evapotranspiration (PET) for the Christians Creek watershed (table 3). Daily minimum and maximum temperature data were collected from SSTP. Missing temperature data were supplemented with temperature data collected at the Dale Enterprise weather station. Dew-point temperature and percent cloud-cover data were collected from the Lynchburg Regional Airport. Collection of percent cloud-cover data at the airport ended June 1996, so percent cloud-cover data for the period July 1996–December 1997 were obtained from Quantico Marine Corp Air Station. Wind-speed data required for calculating PET were collected from Elkins–Randolph Airport, Elkins, W.Va. Daily PET values were calculated using the Hamon equation (Hamon, 1961), which is part of the USEPA software package WDMUtil (U.S. Environmental Protection Agency, 2001). The average of the annual PET values was compared and calibrated to average annual evaporation from a Class A Pan (Kohler and others, 1959). A Class A Pan coefficient of 76 percent was applied, in the model, to the calculated PET values because values of evaporation from a Class A

Pan generally are higher than actual evapotranspiration (Kohler and others, 1959). Daily values of PET were disaggregated to hourly values using WDMUtil.

Streamflow data for Christians Creek, for the period October 1, 1990–September 30, 1999, were collected by the USGS every 15 minutes at the Christians Creek at Fishersville stream gage (USGS station number 01624800) (fig. 1; table 3). Hourly streamflow values were used for the streamflow simulation. Average annual streamflow for the period October 1, 1990–September 30, 1997 (water years 1991-97), was 75.4 ft^3/s with a maximum average annual streamflow of 113.2 ft^3/s during water year 1996 and a minimum average annual streamflow of 41.5 ft^3/s during water year 1995.

All model input (meteorological, streamflow, and water-quality) time-series datasets were loaded into the Watershed Data Management format (WDM) using the computer program WDMUtil. WDMUtil provides the functionality of summarizing, listing, and graphing datasets in the WDM format. Input datasets can be retrieved in HSPF from and output datasets (simulated streamflow and fecal coliform bacteria) written to the WDM file.

Calibration Approach

The objective of the streamflow modeling effort was to simulate the observed water budget and hydrologic response in the Christians Creek watershed. The 6-year simulation period extended from April 1, 1991, to September 30, 1997, and included a 4-year calibration and a 2-year verification period. Key steps in the development of the calibrated model of streamflow for the Christians Creek watershed included collection of historical meteorological and streamflow data, determination of the effective impervious area, calibration of hydraulic parameters, and evaluation of the model results.

A suite of physically based hydraulic parameters governs the streamflow simulation in HSPF. These hydraulic parameters are categorized as fixed and adjusted parameters. Fixed hydraulic parameters can be measured or are well documented in the literature and can be used with a high degree of confidence, such as the length, slope, width, depth, and roughness of a stream channel. Fixed hydraulic parameters are held constant in HSPF during model calibration. Adjusted hydraulic parameters are highly variable in the environment or are immeasurable, such as the infiltration rate and the extent of the lower zone storage area. These adjusted hydraulic parameters represent the hydrologic

Table 4. Initial streamflow model parameters and percent imperviousness in six subwatersheds represented in the streamflow model for Christians Creek, Augusta County, Virginia

[HRU, Hydrologic Response Unit; see table 1 for definitions of parameters; U, Urban; R, Residential; P, Pasture; H, Hayland; C, Cropland; F, Forest; UI, Urban impervious; PI, Pastureland impervious; –, not applicable]

HRU	Imperviousness (percent)	AGWETP	AGWRC (1 per day)	BASETP	DEEPFR	INFILT (inches per hour)	INTFW	IRC (1 per day)	KVARY (1 per inch)	LZETP	LZSN (inches)	UZSN (inches)
U	–	0.00	0.985	0.00	0.50	0.02	0.40	0.60	0.00	0.20	7.00	0.50
R	–	.00	.985	.00	.50	.02	.40	.60	.00	.20	7.00	.50
P	–	.00	.985	.00	.50	.02	1.00	.60	.00	.20	10.00	.80
H	–	.00	.985	.00	.50	.03	.80	.60	.00	.20	8.50	.60
C	–	.00	.985	.00	.50	.03	.80	.60	.00	.20	8.50	.60
F	–	.00	.985	.00	.50	.09	1.00	.65	.00	.20	9.50	.70
UI	38	–	–	–	–	–	–	–	–	–	–	–
PI	1	–	–	–	–	–	–	–	–	–	–	–

transport and storage components in HSPF; each parameter is adjusted/calibrated until simulated streamflow closely represents observed streamflow. Eleven parameters were adjusted to obtain a calibrated model of streamflow for the Christians Creek watershed (table 4).

Results from the streamflow model were evaluated for both the calibration and verification periods. The period from October 1, 1993, to September 30, 1997 (water years 1994-97), was selected for calibration because of the observed variability in average annual streamflow. The largest (113.2 ft^3/s) and smallest (41.5 ft^3/s) amount of average annual streamflow occurred during this period. Calibration over this period ensures that the streamflow model will account for this increased hydrologic variability. Results from the model calibration were evaluated on the basis of comparisons between simulated and observed streamflow with respect to water budget (total runoff volume), high-flow and low-flow distribution (comparison of low-flow and high-flow periods), stormflow (comparison of stormflow volume, peak, and recession), and season (seasonal runoff volume). These comparisons were performed using Expert System for the Calibration of the Hydrological Simulation Program–FORTRAN (HSPEXP) (Lumb and others, 1994). Seven calibration criteria, expressed as a percent difference, were established in HSPEXP to aid in the evaluation of simulated and observed runoff:

Calibration criterion	Percent difference
Total annual runoff	10
Highest 10-percent flows	10
Lowest 50-percent flows	15
Winter runoff	15
Spring runoff	15
Summer runoff	15
Fall runoff	15

Finally, graphs were used to compare simulated and observed streamflow with respect to daily and hourly streamflow, flow-duration curves, and residuals.

The calibrated streamflow model was verified by simulating streamflow during the period from April 4, 1991, to September 30, 1993, using the adjusted hydrologic parameters obtained during model calibration. Model verification was performed once and was not used in the iterative calibration process. Results from model verification were evaluated following the same protocol as described for evaluation of the calibrated model results.

Fecal Coliform Model

After the streamflow model is calibrated, the next step in generating a watershed-scale bacterial transport model is to simulate the transport of bacteria from the land surface, to the stream channel, and through the stream network. In HSPF, this is accomplished by linking the fecal coliform simulation to the streamflow simulation. The following sections summarize the simulation of fecal coliform bacteria in the PERLND, IMPLND, and RCHRES modules. Additional information regarding the simulation of fecal coliform bacteria using HSPF can be found in Bicknell and others (1997).

Pervious and Impervious Land Segments

The PQUAL module is used to simulate the transport of fecal coliform bacteria from pervious land segments. Similar to the PWATER module, PQUAL simulates storages and fluxes of bacteria along three flow paths: overland flow, interflow, and base flow. There are 11 model parameters used to simulate fecal coliform bacteria (table 5). Collectively, these parameters govern the total fecal coliform loading from each HRU to a given stream reach.

The processes by which the transport of fecal coliform bacteria is simulated can be split into two categories: surface and subsurface (interflow and base flow) (fig. 4). The surface processes begin with deposition of feces containing fecal coliform bacteria onto the land surface by numerous sources in the watershed (people, pets, livestock, and wildlife). Fecal coliform deposition is established by the accumulation rate (ACCUM). These bacteria are stored on the surface (SQO) and are allowed to accumulate until the storage limit (SQOLIM) is reached. Bacteria are removed from surface storage by either die-off or washoff. The removal rate (REMQOP) of the stored bacteria through die-off is defined by the ratio of the accumulation rate (ACCUM) and the storage limit (SQOLIM). Bacteria remaining in storage are removed through washoff by overland flow. The amount of bacteria removed from surface storage (SOQUAL) during a given storm event is controlled by both the amount of overland flow generated (SURO) and the susceptibility of the bacteria to washoff by overland flow (WSFAC). SURO is identified for each HRU during the hydrologic calibration. WSFAC is a function of the rate of runoff that results in 90 percent washoff of stored fecal coliform bacteria in a given hour (WSQOP). Below are the governing equations for the release of fecal coliforms from storage on the land surface to the receiving stream channel:

$$SOQUAL = SQO*(1 - e^{(-SURO*WSFAC)}) \tag{2}$$

$$WSFAC = \frac{2.30}{WSQOP} \tag{3}$$

where SOQUAL is the amount of fecal coliform bacteria washed off the land surface (number of colonies/acre/interval),

SQO is surface storage of fecal coliform bacteria (number of colonies/acre),

SURO is the total amount of surface runoff (in/interval),

WSFAC is susceptibility of fecal coliform bacteria to washoff (per inch), and

WSQOP is the rate of surface runoff that results in 90 percent washoff of fecal coliform bacteria in 1 hour (in/hr).

In the simulation of the transport of fecal coliform bacteria through the subsurface, PQUAL allows for the storage and release of bacteria from interflow (IQO) and active ground-water (AQO) storages. The subsurface transport processes represented are simplified considerably compared to those used to represent surface transport. A concentration of fecal coliform bacteria is assigned to both IQO and AQO and is held constant during the simulation. These bacteria are transported to the stream channel with interflow and base flow. The total volume of interflow and base flow that discharges to the stream channel is established during the streamflow model calibration.

IQUAL is used to simulate the transport of fecal coliform bacteria from impervious land segments. The IQUAL module only simulates surface washoff of fecal coliform bacteria because impervious land segments do not have a subsurface component. The transport processes and governing equations (2, 3) used in IQUAL are identical to those used in the surface washoff component of PQUAL. Generally, bacteria stored on an impervious land segment are more susceptible to washoff than those stored on pervious land segments; thus, WSFAC for impervious land segments is greater than WSFAC for pervious land segments.

Table 5. Parameters used in the simulation of the transport and storage of fecal coliform bacteria in Christians Creek, Augusta County, Virginia

[ft^3, cubic feet]

Parameter	Definition	Unit
ACCUM	Accumulation rate of fecal coliform bacteria on the land surface.	number of colonies per acre per day
AOQUAL	Transport of fecal coliform bacteria through base flow (ground-water discharge).	number of colonies per day
AQO	Storage of fecal coliform bacteria in active ground water.	number of colonies per ft^3
IOQUAL	Transport of fecal coliform bacteria through interflow.	number of colonies per day
IQO	Storage of fecal coliform bacteria in interflow.	number of colonies per feet
REMQOP	Removal rate (die-off) for fecal coliform bacteria stored on the land surface. Removal rate is based on the ratio of ACCUM/SQOLIM.	1 per day
SOQUAL	Transport of fecal coliform bacteria through overland flow.	number of colonies per acre per day
SQO	Storage of fecal coliform bacteria on the land surface.	number of colonies per acre
SQOLIM	Asymptotic limit for the storage of fecal coliform bacteria on the land surface if no washoff occurs.	number of colonies per acre
WSFAC	Susceptibility of fecal coliform bacteria to washoff. Susceptibility is defined by 2.30/WSQOP.	per inch
WSQOP	Rate of surface runoff that results in 90-percent washoff of the stored fecal coliform bacteria in one hour.	inches per hour

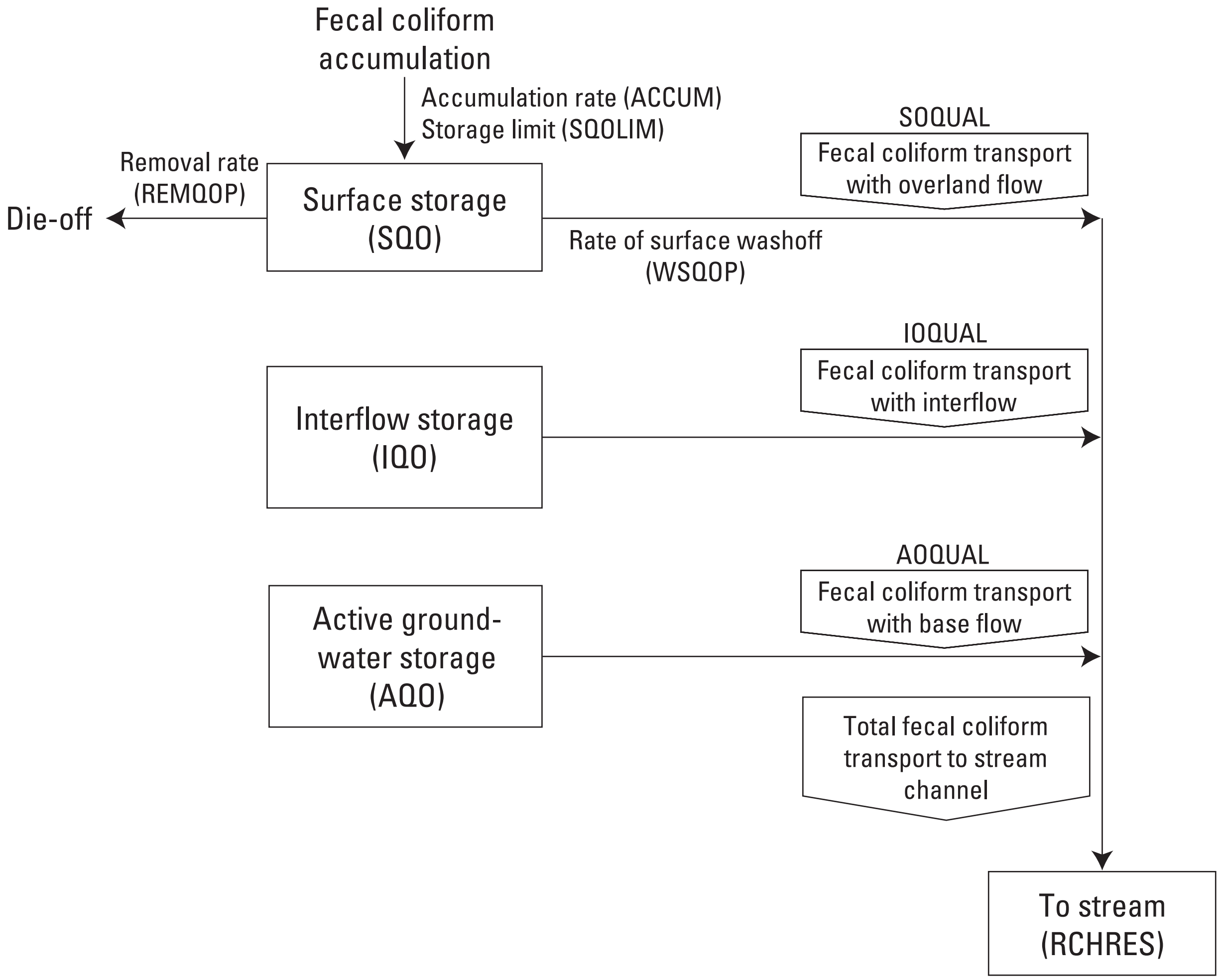


Figure 4. Routing processes represented by the Hydrological Simulation Program-FORTRAN for the simulation of fecal coliform bacteria transport in Christians Creek, Augusta County, Virginia. (See table 5 for definition of fecal coliform bacteria transport and storage parameters.)

Stream Channels

GQUAL is the component in the RCHRES module used to simulate the transport of fecal coliform bacteria through the channel network. Bacteria are routed to the simulated stream channels from the various PERLND and IMPLND HRUs, point source inputs (sewage-treatment plants and instream animals), and upstream stream segments. These bacteria enter the simulated stream segment at a single upstream point and are either transported to the next downstream stream segment or are removed through die-off. The portion of bacteria removed from the simulated stream channel through die-off is based on a first-order decay rate of 1.1 day^{-1} (U.S. Environmental Protection Agency, 1985) and is determined by the following equations:

$$DDQALT = DQAL*(1 - e^{(-KGEN)})*VOL \qquad (4)$$

$$KGEN = (KGEND)(THGEN)^{(TW20)} \qquad (5)$$

where DDQALT is the number of bacteria removed through die-off (number of colonies/interval),

DQAL is the concentration of bacteria for the time interval (number of colonies/100 mL),

KGEN is the generalized first-order decay rate corrected for temperature (number of colonies/interval), and

VOL is the volume of water in the reach (ft^3).

KGEND is the base first-order decay rate (number of colonies/interval),

THGEN is the temperature correction parameter, dimensionless, and

TW20 is the temperature of the water (°C) for interval minus 20.

Limitations of the Fecal Coliform Model

The most critical limitation associated with the fecal coliform model is that fecal coliform bacteria are simulated as a dissolved constituent. Fecal coliform bacteria, however, are particulate constituents and are deposited and resuspended once delivered to the active stream channel. The transport mechanisms associated with deposition and resuspension are not simulated explicitly. However, mechanisms that mimic deposition and resuspension are simulated through interflow and base-flow pathways (see Fecal Coliform Bacteria in the Subsurface).

Point and Nonpoint Source Representation

A key step in simulating the transport of fecal coliform bacteria is to determine the total amount of bacteria deposited on the land surface (representing nonpoint sources) or deposited directly in the stream channel (representing point sources). For this study, the total amount of bacteria deposited by each of the dominant sources of fecal coliform bacteria was estimated. This information was the primary input dataset for the fecal coliform model; the fecal coliform deposition information is analogous to rainfall data used in the runoff model. The following sections explain how the fecal coliform deposition rate was established for the various point sources (for example, STPs) and nonpoint sources (people, pets, livestock, and wildlife) within the Christians Creek watershed.

There are six permitted point source dischargers of fecal coliform bacteria in the Christians Creek watershed (table 6). Three are STPs, each of which is permitted by DEQ to release treated wastewater to Christians Creek or associated tributaries. According to the permit, this wastewater may not contain fecal coliform bacteria concentrations that exceed 200 col/100 mL. The maximum permitted discharge rate and fecal coliform concentration were used to represent each of these STPs in the Christians Creek watershed model. These STPs were represented as a continuous and direct supply of water and bacteria to the respective simulated stream channel. The combined total annual load from the STPs is 2.33×10^{12}col/year. There also are 12 private permitted dischargers, including 9 residences and 3 small businesses, in the watershed (table 7). Combined, these private dischargers contribute 3.32×10^{10}col/year to Christians Creek.

Most of the fecal coliform bacteria in Christians Creek are derived from and represented as nonpoint sources. These bacteria are deposited on the land surface by many different sources (people, pets, livestock, and wildlife) and subsequently are transported to the stream network with rainfall runoff. Two critical pieces of information must be obtained to simulate the transport of fecal coliform bacteria derived from nonpoint sources using HSPF. First, the dominant sources of fecal coliform bacteria in the watershed must be identified. A survey was conducted of fecal coliform sources in the Christians Creek watershed, and 13 sources were identified as potentially dominant and represented in the model. These 13 sources are beavers, cats, cattle, deer, dogs, ducks, geese, horses, humans, muskrats, poultry, raccoons, and sheep. Second, the total daily amount of fecal coliform bacteria deposited on the land surface or directly in streams (straight pipes, cattle in streams, and beaver) by each of the identified sources must be determined for both pervious and impervious land segments.

General Quantification of Fecal Coliform Bacteria

The amount of fecal coliform bacteria deposited on the land surface daily is represented by ACCUM in HSPF. Every source represented in the model has a specific fecal coliform accumulation rate. The following equation is used to calculate ACCUM for each fecal coliform source:

$$ACCUM = \frac{(Fprod*FCden)POPN}{HAB} \qquad (6)$$

where ACCUM is the fecal coliform bacteria accumulation rate (number of colonies/acre/day),

Fprod is the feces produced per day (g/day),

Table 6. Permitted point-source dischargers of fecal coliform bacteria in Christians Creek, Augusta County, Virginia, 1992-97

Permit number	Owner	Facility	Discharge rate (million gallons per day)	Fecal coliform limit (number of colonies per 100 milliliters)	Annual fecal coliform load (number of colonies per year)
VA0025291	Augusta County Service Authority	Fishersville Sewage Treatment Plant	0.7	200	1.94×10^{12}
VA0022306	Augusta County Service Authority	Staunton Plaza Sewage Treatment Plant	.09	200	2.49×10^{11}
VA0022292	Augusta County Service Authority	Brookwood Interchange	.03	200	8.32×10^{10}
VA0020427	Augusta County School Board	Rivershead High School	.014	200	3.88×10^{10}
VA0089061	Woodlawn Village L.L. Corp.	Woodlawn Village Mobile Home Park	.007	200	1.94×10^{10}
VA0086738	Southern States Coop, Inc.	Southern States Coop	0	200	0
Total					2.33×10^{12}

Table 7. Private permitted point-source dischargers of fecal coliform bacteria in Christians Creek, Augusta County, Virginia

Permit number	Discharge rate (gallons per day)	Fecal coliform limit (number of colonies per 100 milliliters)	Annual fecal coliform load (number of colonies per year)
VAG401655	1,000	200	2.76×10^9
VAG401967	1,000	200	2.76×10^9
VAG401968	1,000	200	2.76×10^9
VAG401082	1,000	200	2.76×10^9
VAG401138	1,000	200	2.76×10^9
VAG401159	1,000	200	2.76×10^9
VAG401195	1,000	200	2.76×10^9
VAG401203	1,000	200	2.76×10^9
VAG401443	1,000	200	2.76×10^9
VAG401449	1,000	200	2.76×10^9
VAG401896	1,000	200	2.76×10^9
VAG401969	1,000	200	2.76×10^9
Total			3.32×10^{10}

FCden is the number of fecal coliform bacteria per gram of feces produced (number/g),

POPN is the population size, dimensionless, and

HAB is the habitat area (acres).

The calculation of ACCUM is based on values of Fprod, FCden, HAB, and POPN that are source specific, and selection of these values is challenging. Information on Fprod and HAB generally is well documented for individual species. Therefore, single values of Fprod and HAB are used and held constant throughout the entire modeling effort. Values of FCden and POPN, however, generally are more variable and poorly documented compared to values of Fprod and HAB. For example, dog, cat, and human feces have measured FCden ranges of 4.1×10^6 col/g to 4.3×10^9 col/g; 8.9×10^4 col/g to 2.6×10^9 col/g; and 1.3×10^5 col/g to 9.0×10^9 col/g, respectively (Mara and Oragui, 1981). This wide range in measured values of FCden is typical of most of the sources represented in the model; therefore, considerable uncertainty is associated with choosing a single value of FCden to represent a given species. Additionally, exact population numbers commonly are unknown for the human, pet, and wildlife populations, and the proportion of the population that contributes to the instream fecal coliform load also is unknown. Because of the uncertainty associated with values of FCden and POPN, two decision rules were established that limit the number of parameters adjusted while refining ACCUM for each source:

(1) When the population size for a given source is well documented, then that value will be used and held constant.

(2) When the population size for a given source is unknown, POPN will be treated as an adjusted parameter and potentially modified during the model-calibration process while FCden is held constant.

Under the first decision rule, FCden will be treated as an adjusted variable and potentially modified during the model-calibration process. Adjustments to FCden account for the uncertainty associated with fixed values of Fprod, POPN, and HAB. Under the second decision rule, adjustments to POPN account for the uncertainty associated with the fixed values of Fprod, FCden, and HAB. The resulting POPN value, following calibration, will be identified as an “effective” value that accounts for the uncertainty associated with the fixed values of Fprod, FCden, and HAB.

In HSPF, the total accumulation rate of fecal coliform bacteria on the land surface is bounded by a storage limit (SQOLIM). This storage limit enables the model to account for the natural die-off of bacteria stored on the land surface. For this study, the storage limit was set to 9 times the accumulation rate, which represents a decay rate of 0.1 day^{-1} (U.S. Environmental Protection Agency, 1985).

Source-Specific Quantification of Fecal Coliform Bacteria

The quantification of fecal coliform bacteria generated by the various sources within the Christians Creek watershed is documented in the following section. The sources described in this section are humans, dogs, cats, beef cattle, dairy cattle, heifers, broilers, turkeys, horses, sheep, deer, geese, ducks, raccoons, muskrats, and beavers. These sources are described with respect to their contribution to the pervious and impervious land segments within the basin.

Pervious Land Segments

The Christians Creek watershed has a human population of approximately 12,000 (1990 Census). Within the watershed, many pathways can allow human-derived fecal coliform bacteria to enter Christians Creek. These pathways include failing septic systems, overflowing sewer lines, leaking sewer lines, and straight pipes (direct discharge of untreated sewage from private residences), the cumulative effect of which was represented by a land application of human waste. The fecal coliform bacteria accumulation rate for the land-applied bacteria was calculated using equation 6. The values used to calculate the initial accumulation rate are in table 8. On average, one person generates approximately 150 g of feces per day (Geldreich and others, 1962) and an estimated 4.66×10^8 col/g of human feces (Mara and Oragui, 1981). The initial population value (POPN) used was based on an estimated septic-system failure rate of 15 percent, which is consistent with failure rates determined for nearby communities (Virginia Polytechnic Institute and State University, 2000). In the Christians Creek watershed, 2,950 houses have septic systems. The average household occupancy rate for Augusta County is 2.69 people (1990 Census). POPN is the most uncertain value in equation 6 and, therefore, is adjusted during the model calibration process. These bacteria then are distributed over the residential land type (HAB) (table 8).

Straight pipes were represented as point sources in HSPF. Three factors were used to estimate the number of straight pipes in each subwatershed: the number of houses in each subwatershed, the age of each house, and the proximity of each house to the nearest stream. The number of houses was identified by using the emergency-911 database for Augusta County, Va., which was provided as a GIS coverage by the county. These houses were placed in three possible age categories (pre-1964, 1964-84, and post-1984). The selection of the age categories was based on two versions of USGS 7.5-minute topographic maps. The first series of topographic maps were derived from 1964 aerial photography and were photo-revised in 1984. Therefore, houses represented on the 1984 maps would have been built between 1964 and 1984. The proximity of each house to the nearest stream was identified using the emergency-911 coverage. The total number of houses within 150 ft of a stream was identified. Ten percent and 2 percent of the houses identified to be within 150 ft of a stream, in the pre-1964 and 1964-84 age categories, respectively, were assumed to have a straight pipe (Virginia Polytechnic Institute and State University, 2000). Based on the outlined methodology, there are an estimated number of four straight pipes within the Christians Creek watershed (table 8). The estimated number of fecal coliform bacteria discharged from each straight pipe is based on the occupancy for a single-family residence of 2.69 people and the daily per capita fecal coliform production rate of 6.99×10^{10} col/day (table 8).

Fecal coliform bacteria derived from dogs were represented as a land application to both urban and residential land types. The accumulation rate for these bacteria was calculated using equation 6. Initial values used to calculate ACCUM are listed in table 9. On average, one dog generates 450 g of feces per day (Weiskel and others, 1996), and an estimated 4.11×10^6 col/g of feces (Mara and Oragui, 1981). The initial value for the total number of dogs in the watershed was based on the estimate of one dog per three people. This estimate was refined further to account for the approximately 20 percent of dog waste that is picked up and disposed of. Additionally, 10 percent of the dog waste was assumed to be deposited on impervious surfaces such as parking lots and roads. The POPN value in table 9 represents the initial estimated number of dogs whose feces are deposited outdoors and are picked up and disposed of. Because the actual number of dogs in the watershed is unknown, POPN is treated as a fitted value during the model-calibration process.

Fecal coliform bacteria derived from cats were represented as a land application to both urban and residential land types. The accumulation rate for these bacteria was calculated using equation 6. Initial values used to calculate ACCUM are listed in table 9. On average, one cat generates 20 g of feces per day (Jutta Schneider, Virginia Department of Conservation and Recreation, written commun., 2000), and an estimated 1.49×10^7 col/g of feces (Mara and Oragui, 1981). The initial value for the total number of cats in the watershed was based on an estimate of two cats per three people. It was assumed that 70 percent of the estimated number of cats deposits their feces outdoors. The POPN value in table 9 represents the initial estimated number of cats that deposit their feces outdoors. Because the actual number of cats that deposit their feces outdoors is unknown, POPN is treated as a fitted value during the model-calibration process.

There are approximately 10,000 beef cattle, 1,650 dairy cattle, and 2,100 heifers in the Christians Creek Watershed. Each of these cattle types has different estimated daily fecal production rates (American Society of Agricultural Engineers, 1998) and associated fecal coliform densities (Mara and Oragui, 1981) (table 10). The fecal coliform bacteria derived from cattle feces can be transported to Christians Creek along three possible pathways: (1) Feces generated while cattle are confined are stored and later distributed over the various croplands in the watershed, and then transported to the stream network with surface runoff. (2) Feces are deposited directly on the pastureland by grazing cattle, and then transported to the stream network through surface runoff. (3) Feces are deposited directly in Christians Creek and associated tributaries by cattle standing in these streams. Each of these three pathways is represented in HSPF.

Table 8. Initial values of the total amount of feces produced daily and fecal coliform bacteria per gram of feces generated by the human population in the residential hydrologic response unit represented in the fecal coliform model, Christians Creek, Augusta County, Virginia

[Fprod, feces produced per day; FCden, fecal coliform bacteria per gram of feces; POPN, population size; HAB, habitat area; –, not applicable]

Subwatershed[1]	Fprod (grams)	FCden	POPN (number of humans)	HAB (acres)
		Human–land applied		
1	150	4.66×10^8	209	289
2	150	4.66×10^8	135	206
3	150	4.66×10^8	260	309
4	150	4.66×10^8	172	485
5	150	4.66×10^8	253	808
6	150	4.66×10^8	161	261
		Human–straight pipes		
1	150	4.66×10^8	3	–
2	150	4.66×10^8	3	–
3	–	–	–	–
4	150	4.66×10^8	3	–
5	–	–	–	–
6	150	4.66×10^8	3	–

[1]See figure 3 for location of subwatersheds.

Table 9. Initial values of the total amount of feces produced daily and fecal coliform bacteria per gram of feces generated by the dog and cat populations in the urban and residential hydrologic response units represented in the fecal coliform model, Christians Creek, Augusta County, Virginia

[Fprod, feces produced per day; FCden, fecal coliform bacteria per gram of feces; POPN, population size; HAB, habitat area]

Subwatershed[1]	Fprod (grams)	FCden	POPN (number)		HAB (acres)	
			Residential	Urban	Residential	Urban
			Dogs			
1	450	4.11×10^6	368	199	289	414
2	450	4.11×10^6	238	129	206	153
3	450	4.11×10^6	458	248	309	495
4	450	4.11×10^6	303	164	485	1,244
5	450	4.11×10^6	446	242	808	196
6	450	4.11×10^6	283	153	261	429
			Cats			
1	20	1.49×10^7	654	393	289	414
2	20	1.49×10^7	423	254	206	153
3	20	1.49×10^7	814	490	309	495
4	20	1.49×10^7	537	324	485	1,244
5	20	1.49×10^7	792	477	808	196
6	20	1.49×10^7	502	302	261	429

[1]See figure 3 for location of subwatersheds.

Table 10. Initial values of the total amount of feces produced daily and fecal coliform per gram of feces generated by dairy cattle, beef cattle, and heifers represented in the fecal coliform model, Christians Creek, Augusta County, Virginia

[Fprod, Feces produced per day; FCden, Fecal coliform bacteria per gram of feces]

Source	Average daily Fprod (grams per day)	FCden
Dairy cattle	54,545	8.18 x 10^5
Beef cattle	20,909	1.87 x 10^6
Heifers	39,091	6.40 x 10^4

Dairy cattle are confined in or around milking parlors between 7.2 and 18.0 hr/day during the summer and winter months, respectively (table 11). The feces generated by confined dairy cattle are collected and stored in anaerobic lagoons. Fecal coliform bacteria stored in anaerobic lagoons are subject to die-off; the die-off rate was simulated using the equation

$$C_t = C_o e^{-Kt} \tag{7}$$

where C_t is the fecal coliform bacteria load at time t,

C_o is the initial fecal coliform bacteria load,

t is the time in days, and

K is the first order decay rate (day^{-1}).

C_o was set to the number of fecal coliform bacteria produced annually by dairy cattle in confinement. The time (t) these bacteria were stored was set to 100 days, which represents the average storage capacity of dairy lagoons in the Christians Creek watershed. A decay rate (K) of 0.375 day^{-1} was used to represent the decay rate observed in an anaerobic lagoon (Crane and Moore, 1986). The amount of fecal coliform bacteria remaining after the 100 days of storage (C_t), which then is available for manual application to croplands, is incorporated into equation 6 to determine the accumulation rate per acre of cropland. Because the number of dairy cattle in the watershed is known, FCden is adjusted during the model-calibration process. The percentage of stored dairy waste applied to cropland varies from month to month (table 12); the monthly field application rate and the number of cattle in confinement were represented by means of monthly ACCUM values. The fecal coliform bacteria from the dairy waste applied to cropland are treated as a nonpoint source in the model simulation.

Beef cattle and heifers spend an average of 9.6 hours per day in confinement during the months of December, January, and February (table 13). During these months, the cattle are confined in a small area but are housed in barns where they deposit feces and associated fecal coliform bacteria. The accumulating manure is removed routinely and stored until it can be applied to cropland. Fecal coliform bacteria are subject to die-off during the manure storage phase. Equation 7 was used to determine the total amount of bacteria removed from the stored manure through die-off. C_o was set to the total number of fecal coliform bacteria produced yearly by beef cattle and heifers in confinement. The total time (t) these bacteria were stored was set to 30 days. A decay rate (K) of 0.066 day^{-1} was used to represent the decay rate in an uncovered manure pile (Crane and Moore, 1986). The amount of fecal coliform bacteria remaining after the 30 days of storage (C_t), which then is available for manual application to croplands is incorporated into equation 6 to determine the accumulation rate per acre of cropland. Because the number of beef cattle and heifers in the watershed is known, FCden is treated as an adjusted value during the model-calibration process. The percentage of stored manure applied to cropland varies from month to month (table 14); the monthly field application rate and number of cattle in confinement were represented in the model by means of monthly ACCUM values. The bacteria from the manure applied to cropland are treated as a nonpoint source in the model simulation.

Dairy cattle spend between 5.5 and 15.8 hours per day in the pasture (table 11) while beef cattle and heifers spend between 13.9 and 23.0 hours per day in the pasture (table 13). In the model, pasture is represented by both pasture and hayland, with 80 percent of the cattle distributed on pasture and 20 percent distributed on hayland. These cattle deposit feces with associated fecal coliform bacteria directly onto the pasture. The daily total number of bacteria deposited on the pasture is determined by the time cattle spend in the pasture and the daily fecal coliform production rate. Monthly values of ACCUM were used to represent the monthly

Table 11. Initial values of the total hours per day dairy cattle spend in a given month in the pasture, in confinement, and with access to a stream in the Christians Creek watershed, Augusta County, Virginia

Month	Time (hours)		
	Pasture	Access to stream	Confinement
January	5.5	0.5	18.0
February	5.5	.5	18.0
March	13.4	1.0	9.6
April	15.8	1.0	7.2
May	15.8	1.0	7.2
June	13.8	3.0	7.2
July	13.8	3.0	7.2
August	13.8	3.0	7.2
September	15.8	1.0	7.2
October	15.8	1.0	7.2
November	13.4	1.0	9.6
December	5.5	.0	18.0

Table 12. Percentage of the total stored liquid dairy cattle waste applied to cropland in the Christians Creek watershed, Augusta County, Virginia

Month	Application amount[1] (percent)
January	0.0
February	5.0
March	25.0
April	20.0
May	5.0
June	7.5
July	2.5
August	5.0
September	12.5
October	7.5
November	10.0
December	.0

[1]From Virginia Department of Conservation and Recreation

Table 13. Total hours per day beef cattle and heifers spend in a given month in the pasture, with access to a stream, and in confinement in the Christians Creek watershed, Augusta County, Virginia

Month	Time (hours per day)		
	Pasture	Access to stream	Confinement
January	13.9	0.5	9.6
February	13.9	.5	9.6
March	23.0	1.0	.0
April	23.0	1.0	.0
May	22.5	1.5	.0
June	20.5	3.5	.0
July	20.5	3.5	.0
August	20.5	3.5	.0
September	22.5	1.5	.0
October	23.0	1.0	.0
November	23.0	1.0	.0
December	13.9	.5	9.6

Table 14. Percentage of stored beef cattle and heifer manure and poultry litter applied to cropland in the Christians Creek watershed, Augusta County, Virginia

Month	Application amount[1] (percent)
January	0.0
February	5.0
March	25.0
April	20.0
May	5.0
June	5.0
July	5.0
August	5.0
September	10.0
October	10.0
November	10.0
December	.0

[1]From Virginia Department of Conservation and Recreation

varying number of cattle in the pastures, and the bacteria from the feces deposited directly to the pasture are treated as a nonpoint source in the model simulation.

When stream access is provided, cattle spend an average of 0.5 hr/day during cold months to 3.5 hr/day during warm months (table 13) in and near streams. In order to determine and simulate the total amount of feces that is deposited directly to the stream, the number of cattle with direct access to a stream must be identified. This number is estimated by first identifying the total number of pasture and hayland land segments (pastures) that are bordered by Christians Creek or its major tributaries, Folly Mills Creek, Barterbrook Branch, Goose Creek, and Meadow Run (fig. 1). GIS coverages for land use and stream networks in the Christians Creek watershed revealed that 35 percent of all pastures are bordered by a major stream. The number of cattle in the major streams is determined by the equation

$$Cattle_{Instream} = (Cattle_{Total}(0.35))\left[\frac{T_{Access}}{24}\right] \qquad (8)$$

where $Cattle_{Instream}$ is the number of dairy cattle, beef cattle, or heifers in the stream,

$Cattle_{Total}$ is the total number of dairy cattle, beef cattle, or heifers in the pastures, and

T_{Access} is the estimated time spent in the stream.

In the model, 30 percent of the fecal coliform bacteria generated by $Cattle_{Instream}$ are represented as deposited directly in the stream whereas the remaining 70 percent is allocated to pastures. This 70 percent represents the feces that are deposited near but not directly in the stream channel. The 30 percent that is directly deposited into the stream is represented using monthly values to account for the varying time cattle spend in the stream each month. This direct deposition is represented in the model as a point source.

There are 10,000 broilers and 172,000 turkeys in the Christians Creek watershed. In addition, 3,000 tons of poultry litter are imported annually. The imported litter and the resident broiler and turkey population was represented in the model as combined poultry. A fecal production rate for turkey of 231 g/day (American Society of Agricultural Engineers, 1998) and an estimated fecal coliform density of 1.82×10^9 col/g (Mara and Oragui, 1981) were used to determine the total number of fecal coliform bacteria produced per day. Because the entire poultry population is confined to poultry houses, the generated poultry litter is stored and later applied to cropland. The extent of fecal coliform bacteria die-off during poultry litter storage was determined using equation 7. C_o was set to the total number of fecal coliform bacteria produced yearly by poultry. The time (t) these bacteria were stored was set to 90 days, which is the average poultry litter storage time. A decay rate (K) of 0.08 day^{-1} was used to represent the decay rate observed for poultry litter applied to the soil surface (Giddens and others, 1973). The amount of fecal coliform bacteria remaining (C_t) after the 90 days of storage is incorporated into equation 6 to determine ACCUM. The percentage of stored poultry litter applied to cropland varies from month to month (table 14); the monthly field application rate was represented in the model by means of monthly ACCUM values. Because the number of poultry in the watershed is known, FCden is adjusted during the model-calibration process. The fecal coliform bacteria from the poultry litter applied to cropland are treated as a nonpoint source in the model simulation.

There are 600 horses and 1,100 sheep in the Christians Creek watershed. The average fecal production rate for horses and sheep is 23,182 g/day and 1,091 g/day, respectively (American Society of Agricultural Engineers, 1998). The fecal coliform density assumed for horse feces is 1.81×10^5 col/g (American Society of Agricultural Engineers, 1998) and 1.80×10^5 col/g for sheep (Mara and Oragui, 1981). ACCUM values for horses and sheep are adjusted during the calibration process, as needed, through the FCden parameter. Horses and sheep deposit their waste directly onto pasture. The bacteria applied to pasture are treated as a nonpoint source in the model simulation.

DCR provided information on the numbers and housing of livestock, as well as application rates of liquid dairy waste and manure to cropland and pasture in the Christians Creek watershed (Jutta Schneider, Virginia Department of Conservation and Recreation, written commun., 2000).

The wildlife sources represented in the model are deer, geese, ducks, raccoons, muskrats, and beavers. These sources were selected on the basis of information from the Virginia Department of Game and Inland Fisheries (VDGIF) and watershed surveys performed by the USGS as part of this study. The population of each of these wildlife species was estimated on the basis of habitat area, species density within the habitat area, and seasonal migration (table 15). GIS coverages for animal habitat and land use were used to determine the size of each animal's habitat. For example, Canada geese prefer to be within 300 ft of streams on all land segments except forested; therefore, the total acres of Canada geese habitat is equal to the sum of the acres of all land segments within 300 ft of a stream, except forested, in the habitat area. The population density for geese and ducks increases during the winter months (December, January, and February) because of migration. The amount of fecal coliform bacteria produced daily by each wildlife species (table 16) is used in equation 6 to identify ACCUM for each wildlife species represented in the model. POPN for all wildlife species except deer, and FCden for deer, are adjusted during the model-calibration process. Monthly values of ACCUM are adjusted for geese and ducks in order to account for migration. The feces of all wildlife species except beaver are deposited directly to the land segments in their habitat; therefore, these sources of fecal coliform bacteria are represented in the model as nonpoint sources. Beaver feces are deposited directly in streams and, therefore, are represented as a point source.

Impervious Land Segments

Dogs are the only source in the model that is assumed to deposit feces on impervious surfaces (table 17). Ten percent of the total waste generated by dogs is assumed to fall directly on the impervious portions of the urban land-use type. The bacteria from the feces directly deposited on impervious surfaces are modeled as a nonpoint source. The fecal coliform accumulation rate is calculated using equation 6 and is based on fecal production from 10 percent of the dog population.

Fecal Coliform Bacteria in the Subsurface

The decision to represent fecal coliform bacteria in the subsurface was based primarily on results from intensive monitoring of fecal coliform bacteria during stormflow and base-flow conditions in Christians Creek (Hyer and Moyer, 2003). Data collected by Hyer and Moyer (2003) support two hypotheses regarding the transport of fecal coliform bacteria. First, in addition to the surface runoff, fecal coliform bacteria may be transported along subsurface pathways. Other studies have found that bacteria can infiltrate and move through the shallow subsurface (Rahe and others, 1978; Wright, 1990; Miller and others, 1991; Pasquarell and Boyer, 1995; Howell and others, 1995; Felton, 1996; McMurry and others, 1998). Second, fecal coliform bacteria may be transported by other mechanisms that mimic subsurface pathways, such as resuspension of fecal coliforms from streambed sediments by animals walking in the stream, sloughing of fecal coliforms from the surface of streambed sediments, or advective transport of fecal coliforms from the streambed sediment by ground-water recharge (Goyal and others, 1977; LaLiberte and Grimes, 1982; Burton and others, 1987; Sherer and others, 1988; Marino and Gannon, 1991). These bacteria transport mechanisms were simulated by incorporating the subsurface modules for interflow and base flow.

Interflow represents water that is transported through the shallow subsurface (soil water). The travel time for soil water to reach the stream is greater than water transported as surface runoff; thus, soil water affects the stream hydrograph by decreasing the rate of recession following a storm event. Similarly, fecal coliform bacteria transported with interflow will extend the period of elevated fecal coliform bacteria concentrations following a storm event. Hyer and Moyer (2003) observed elevated fecal coliform concentrations for up to 2 days following storm events in Christians Creek. Fecal coliform bacteria associated with instream suspended sediment may contribute to post-storm elevated fecal coliform concentrations and are represented by simulation of the interflow component. Hyer and Moyer (2003) observed similar post-storm responses for streamflow, suspended sediment, and fecal coliform bacteria. In HSPF, the post-storm response for fecal coliform bacteria concentration was represented by assigning a concentration of 1,500 col/100 mL (424,800 col/ft^3) to interflow. These bacteria were linked to the top four fecal coliform bacteria sources identified by Hyer and Moyer (2003). These sources are cattle, dogs, humans, and poultry.

Base flow, which represents the portion of ground water that enters the stream, is the dominant component of the stream hydrograph during periods of extended dry weather. Fecal coliform bacteria observed during these base flow periods typically are transported through diffuse ground-water input or pathways that mimic this diffuse input, such as resuspension of fecal coliforms from streambed sediments by animals walking in the stream, sloughing of fecal coliforms from the surface of streambed sediments, and advective trans-

Table 15. Initial population values of wildlife sources of fecal coliform bacteria in the fecal coliform model, Christians Creek, Augusta County, Virginia

[POPN, population size; F, Forest; P, Pasture; U, Urban; R, Residential; H, Hayland; C, Cropland]

Wildlife source	Land-use type	Habitat[1]	Population density[2] (number per acre)	POPN (number)
Deer	F, P	Entire watershed	0.040	975
Goose–Summer	U, R, P, H, C	Within 300 feet of streams and ponds	.078	373
Goose–Winter	U, R, P, H, C	Within 300 feet of streams and ponds	.11	526
Duck–Summer	U, R, P, H, C	Within 300 feet of streams and ponds	.047	225
Duck–Summer	F	Within 300 feet of streams and ponds	.016	35
Duck–Winter	U, R, P, H, C	Within 300 feet of streams and ponds	.063	301
Duck–Winter	F	Within 300 feet of streams and ponds	.031	67
Raccoon	F	Within 2,640 feet of streams and ponds	.055	1,083
Raccoon	R, P, H, C	Within 2,640 feet of streams and ponds	.023	1,042
Muskrat	U, R, P, H, C, F	Within 60 feet of streams and ponds	.500	479
Beaver	F	Within 60 feet of streams and ponds	.016	5
Beaver	U, R, P, H, C	Within 60 feet of streams and ponds	.008	5

[1]Paul Bugas, Virginia Department of Game and Inland Fisheries, oral commun., 1999, and U.S. Department of Agriculture, Forest Service, Rocky Mount Research Station, Fire Sciences Laboratory, Fire Effects Information System (January, 2000).
[2]Paul Bugas, Virginia Department of Game and Inland Fisheries, oral commun., 1999.

Table 16. Initial values of the total amount of feces produced daily and fecal coliform bacteria per gram of feces generated by deer, goose, duck, raccoon, muskrat, and beaver represented in the fecal coliform model, Christians Creek, Augusta County, Virginia

[Fprod, feces produced per day; FCden, fecal coliform bacteria per gram of feces]

Wildlife source	Fprod (grams)	FCden
Deer	772	3.30×10^{6}
Goose	225	3.55×10^{6}
Duck	150	4.90×10^{7}
Raccoon	450	1.11×10^{7}
Muskrat	100	2.50×10^{5}
Beaver	200	1.00×10^{3}

Table 17. Initial values of the total amount of feces produced daily and fecal coliform bacteria per gram of feces generated by the dog population in the urban impervious hydrologic response unit represented in the fecal coliform model, Christians Creek, Augusta County, Virginia

[Fprod, feces produced per day; FCden, fecal coliform bacteria per gram of feces; POPN, population size; HAB, habitat area]

Subwatershed[1]	Fprod (grams)	FCden	POPN (number)	HAB (acres)
1	450	4.11×10^6	22	26
2	450	4.11×10^6	14	10
3	450	4.11×10^6	28	32
4	450	4.11×10^6	18	170
5	450	4.11×10^6	27	13
6	450	4.11×10^6	17	27

[1]See figure 3 for location of watersheds.

port of fecal coliforms from the streambed sediment by ground-water inputs. Results from Hyer and Moyer (2003) indicate that bacteria linked to poultry, pet, and other nonpoint sources were present in base-flow samples from Christians Creek. Although the transport mechanism is unknown, nonpoint source signatures in base flow are represented through the ground-water module. In HSPF, a fecal coliform bacteria concentration of 100 col/100 mL (28,320 col/ft^3) was assigned to base flow. These bacteria also were linked to cattle, dogs, humans, and poultry identified by Hyer and Moyer (2003).

Water-Quality Data

DEQ monitors water quality in streams and rivers across the State. One constituent monitored is fecal coliform bacteria, which are derived from the intestinal tract of warm-blooded animals. These bacteria are used as an indicator organism for identifying the presence of fecal contamination and associated pathogens such as *Salmonella* and *Shigella*. The predominant form of fecal coliform bacteria is *Escherichia coli (E. coli).* DEQ collects and analyzes water samples to determine if a particular stream or river is in compliance with the State water-quality standard for fecal coliform bacteria, which is an instantaneous concentration of 1,000 col/100 mL. Sites with fecal coliform bacteria concentrations greater than 1,000 col/100 mL pose a risk to individuals who are in direct contact with the contaminated water because of the increased likelihood of encountering a pathogen (U.S. Environmental Protection Agency, 1986). DEQ has established a lower detection limit of 100 col/100 mL and an upper detection limit of 8,000 col/100 mL for enumeration of fecal coliform bacteria. Therefore, fecal coliform bacteria concentrations reported by DEQ of 100 and 8,000 col/100 mL have an actual concentration of 0–100 col/100 mL or greater than or equal to 8,000 col/100 mL, respectively. DEQ generally collects water-quality samples monthly under low-flow or post stormflow conditions; peak stormflow water-quality samples are not collected routinely.

DEQ collects monthly water-quality samples at two long-term monitoring stations on Christians Creek (fig. 1; table 18). Samples are analyzed for fecal coliform bacteria using the membrane filtration technique. Results of monitoring during 1991-97 show that fecal coliform bacteria concentrations were higher than the State instantaneous water-quality standard in 64.4 percent of the samples taken at the upstream site (Route 831) (fig. 5) and in 33.8 percent of the samples taken at the downstream site (Route 794) (fig. 6). Comparison of the fecal coliform bacteria data from the Route 831 and Route 794 monitoring stations (fig. 7), by means of a two-sided Wilcoxon rank sum test, indicates significantly higher concentrations at the Route 831 station relative to those at the Route 794 station ($p = 0.0004$). Seasonal patterns also were identified in the data (figs. 8-9). Generally, fecal coliform concentrations are higher during the warmer months (April–October) and lower during the cooler months (November–March). This seasonal pattern was more pronounced at the Route 831 station than the Route 794 station and is consistent with the animal practices in the

Table 18. Fecal coliform bacteria concentrations for water-quality samples collected by the Virginia Department of Environmental Quality at two water-quality monitoring stations on Christians Creek, Augusta County, Virginia

Station number[1]	Station name	Latitude Longitude	Period of record	Fecal coliform bacteria concentration, in colonies per 100 milliliters			
				Minimum	Maximum	Mean	Median
1BCST021.76	Route 831	38°03′22″ 79°04′18″	1991-2001	100	8,000	2,526	1,500
1BCST012.32	Route 794	38°07′43″ 78°59′41″	1979-2001	100	8,000	1,205	600

[1]See figure 1 for location of stations.

watershed (increased animal density and activity around the streams during the hot summer months) and possible seasonal differences in bacteria survivorship. Similar seasonal patterns have been observed in other studies of fecal coliform concentrations and loads (Christensen and others, 2001; Baxter-Potter and Gilliland, 1988).

The USGS collected water-quality data for this study at six sites in Christians Creek from March 1999 to October 2000 (Hyer and Moyer, 2003). All stream-water samples were analyzed for the enumeration of fecal coliform bacteria following standard USGS methods for the membrane filtration technique (Myers and Sylvester, 1997). Stream-water samples were collected over the complete range of hydrologic conditions (table 19).

Low-flow samples were collected every 6 weeks at Route 794. Some of these low-flow sampling events were on the recession limbs of storm events. Typically, between four and eight depth-integrated samples were collected during each low-flow sampling event. Consecutive samples were collected at three locations across the stream width (the center of the channel and approximately halfway to each stream bank). The depth-integrated samples were collected at 5-minute intervals, providing a degree of time-integration during each sampling event. Results of the water-quality samples collected under low-flow and recession-flow conditions indicate that 50 percent of the low-flow samples exceeded the State fecal coliform bacteria standard (fig. 10). Recession-flow samples generally had fecal coliform concentrations that were elevated relative to the low-flow samples. The fecal coliform data also exhibited a strong seasonal pattern; higher concentrations were observed during the warmer months (June–September) than during the cooler months (October–May). This seasonal pattern for concentrations of fecal coliform bacteria is consistent with the pattern identified in the historical data.

Stormflow samples were collected during five storm events (September 9, 1999; November 11, 1999; March 20, 2000; April 25, 2000; and June 28, 2000) at Route 794. At least 10 water samples were collected across the storm hydrograph (rising limb, plateau, and falling limb) during each storm event. The fecal coliform concentrations observed during these storm events are elevated considerably relative to the State water-quality standard (fig. 11) and the low-flow concentrations. A large range of concentrations was observed during each storm because sampling was done over the entire hydrograph. Peak fecal coliform concentrations observed during these storms ranged from 23,000 to 730,000 col/100 mL. Elevated fecal coliform concentrations during storm events have been observed in previous studies (Christensen and others, 2001; Bolstad and Swank, 1997). In general, these elevated stormflow concentrations are interpreted as resulting from a combination of a flushing response (whereby fecal coliform bacteria that have been deposited near the stream are washed off the land surface and into the stream) and a resuspension of streambed sediments containing fecal coliform bacteria (Hunter and others, 1992; McDonald and Kay, 1981).

Five continuum sampling sites in addition to Route 794 were established along Christians Creek (fig. 1; table 19). These six sites were sampled three times (March 25, 1999; July 27, 1999; and August 1, 1999) to examine how well the intensive sampling at Route 794 represented the entire watershed. These samples were collected as a single, depth-integrated sample from the approximate center of the stream channel. Additionally, data from these continuum sites provided information on the spatial variability observed in concentrations of fecal coliform bacteria (table 19). These data are con

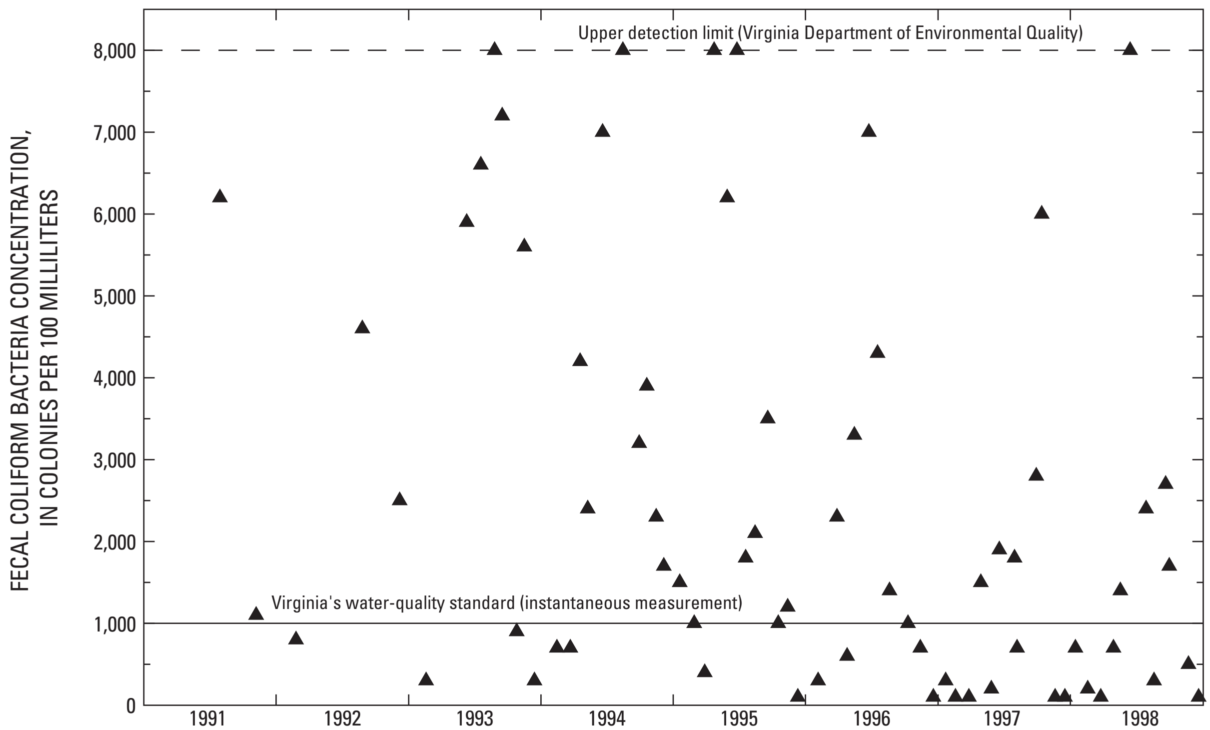


Figure 5. Observed fecal coliform bacteria concentrations for Christians Creek at Route 831, Augusta County, Virginia, from 1991-98. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)

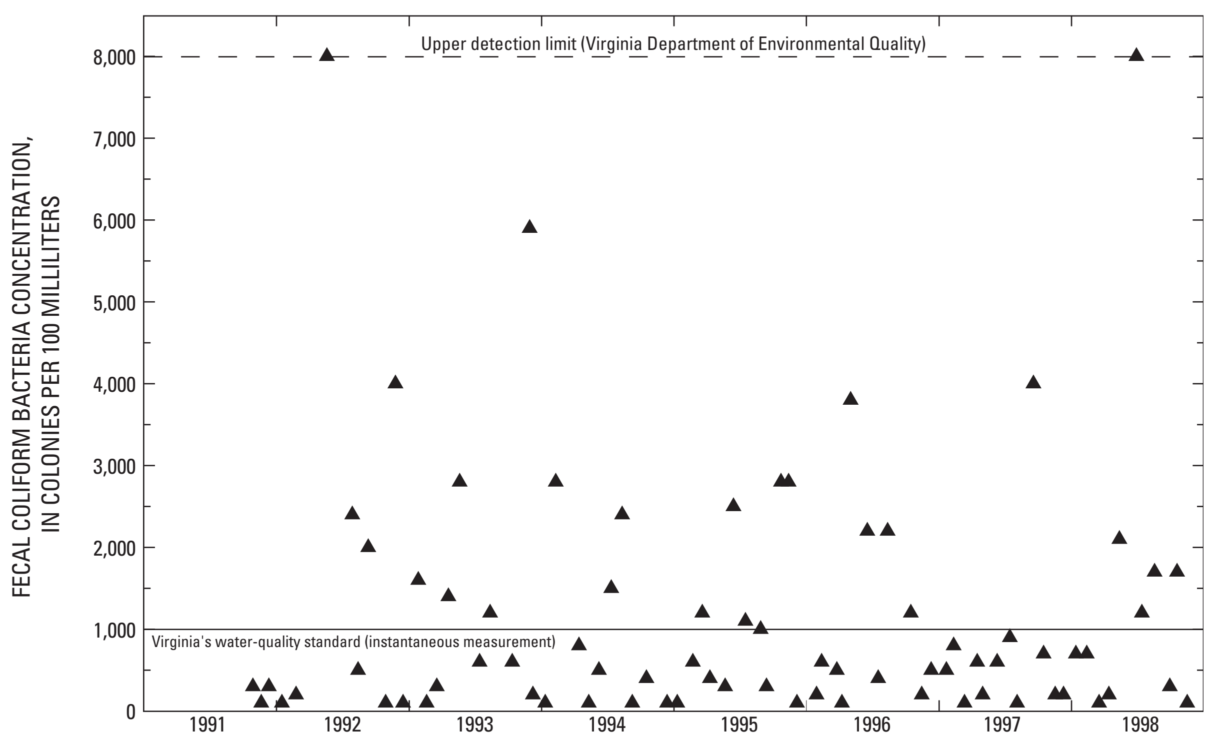


Figure 6. Observed fecal coliform bacteria concentrations for Christians Creek at Route 794, Augusta County, Virginia, from 1991-98. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)

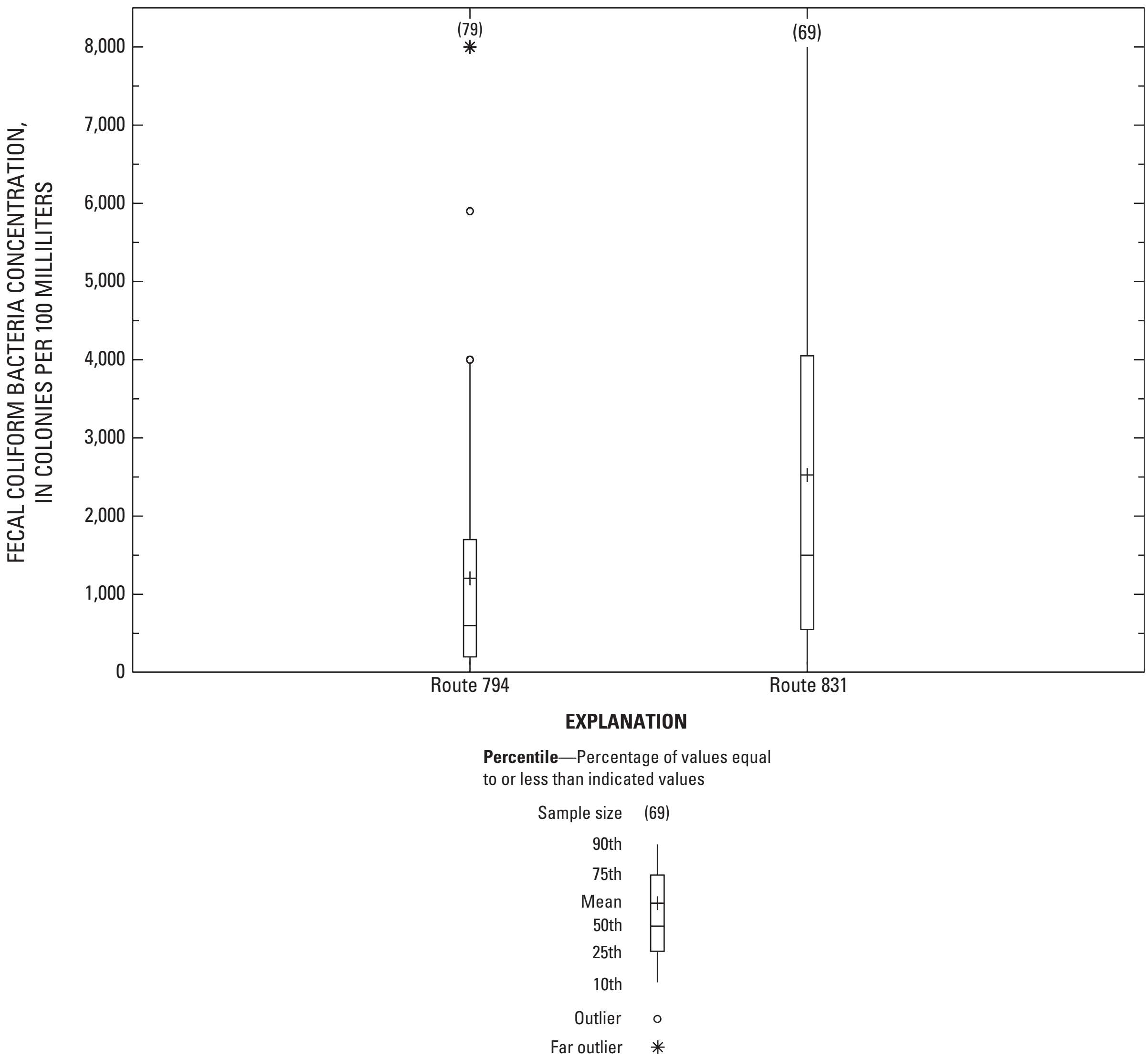


Figure 7. Relation between observed fecal coliform bacteria concentrations for Christians Creek at Route 831 and Route 794, Augusta County, Virginia, 1991-97. (Observed data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)

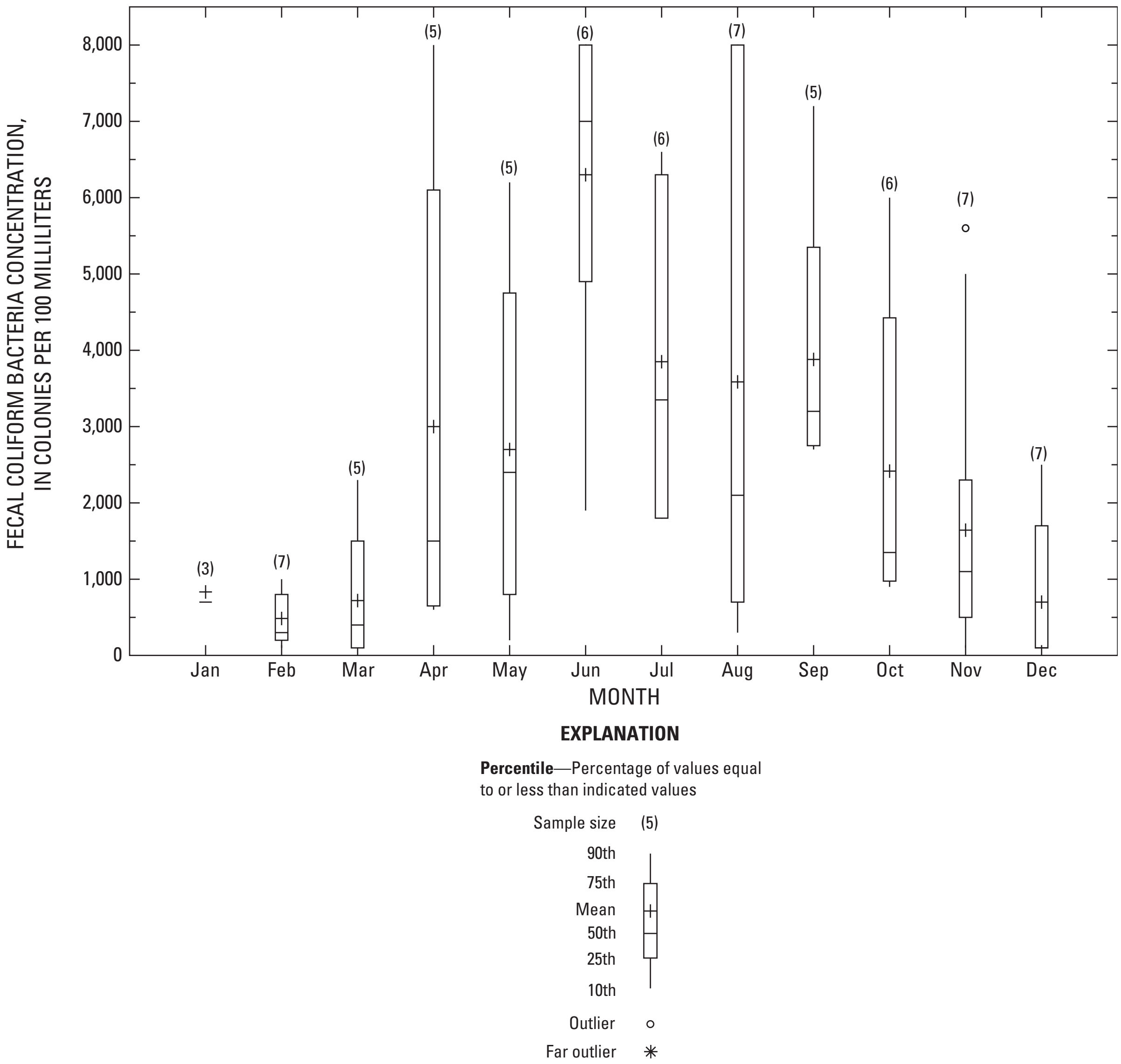


Figure 8. Monthly distribution of observed fecal coliform bacteria concentrations for Christians Creek at Route 831, Augusta County, Virginia,1991-97. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)

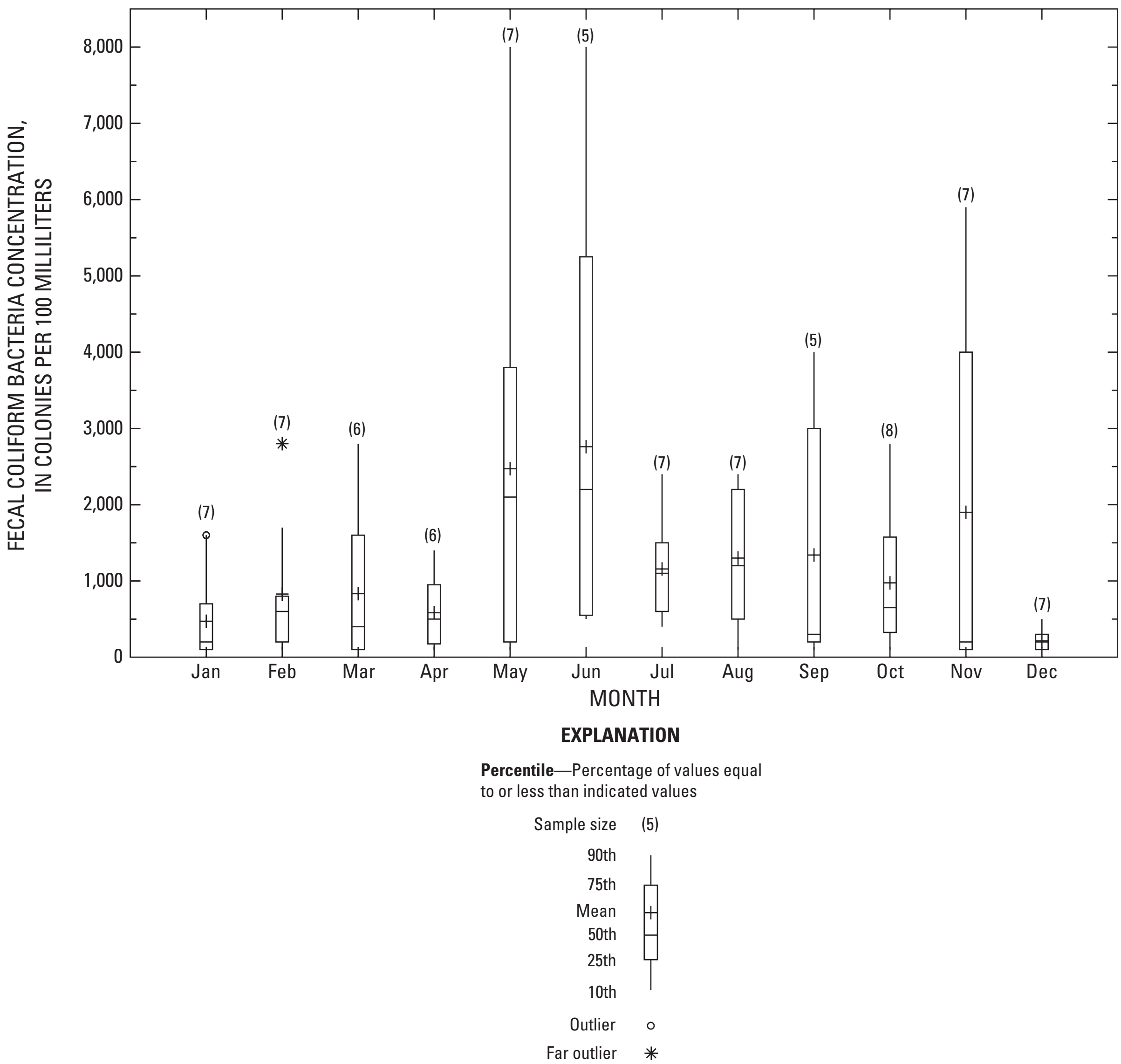


Figure 9. Monthly distribution of observed fecal coliform bacteria concentrations for Christians Creek at Route 794, Augusta County, Virginia1991-97. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)

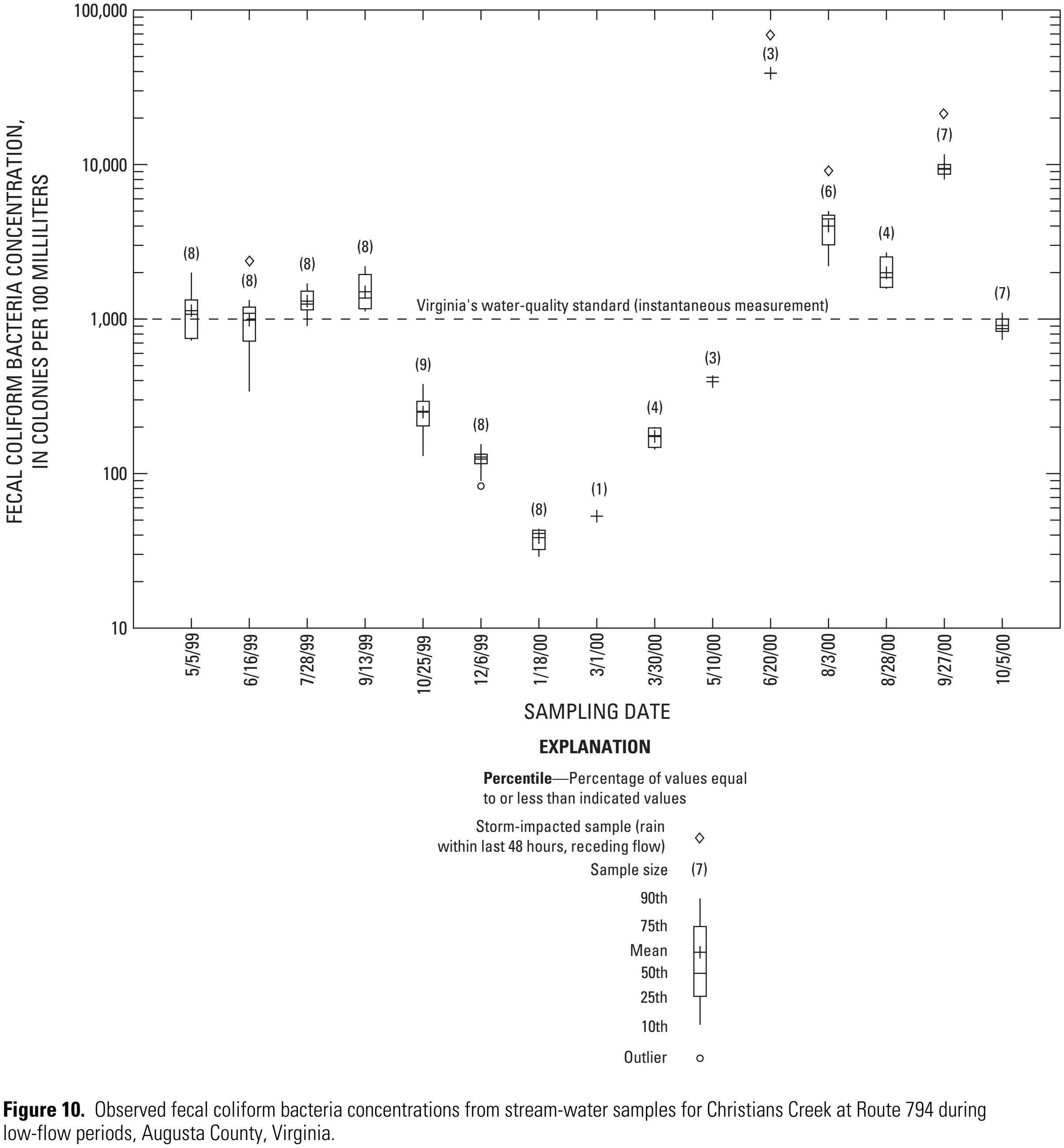


Figure 10. Observed fecal coliform bacteria concentrations from stream-water samples for Christians Creek at Route 794 during low-flow periods, Augusta County, Virginia.

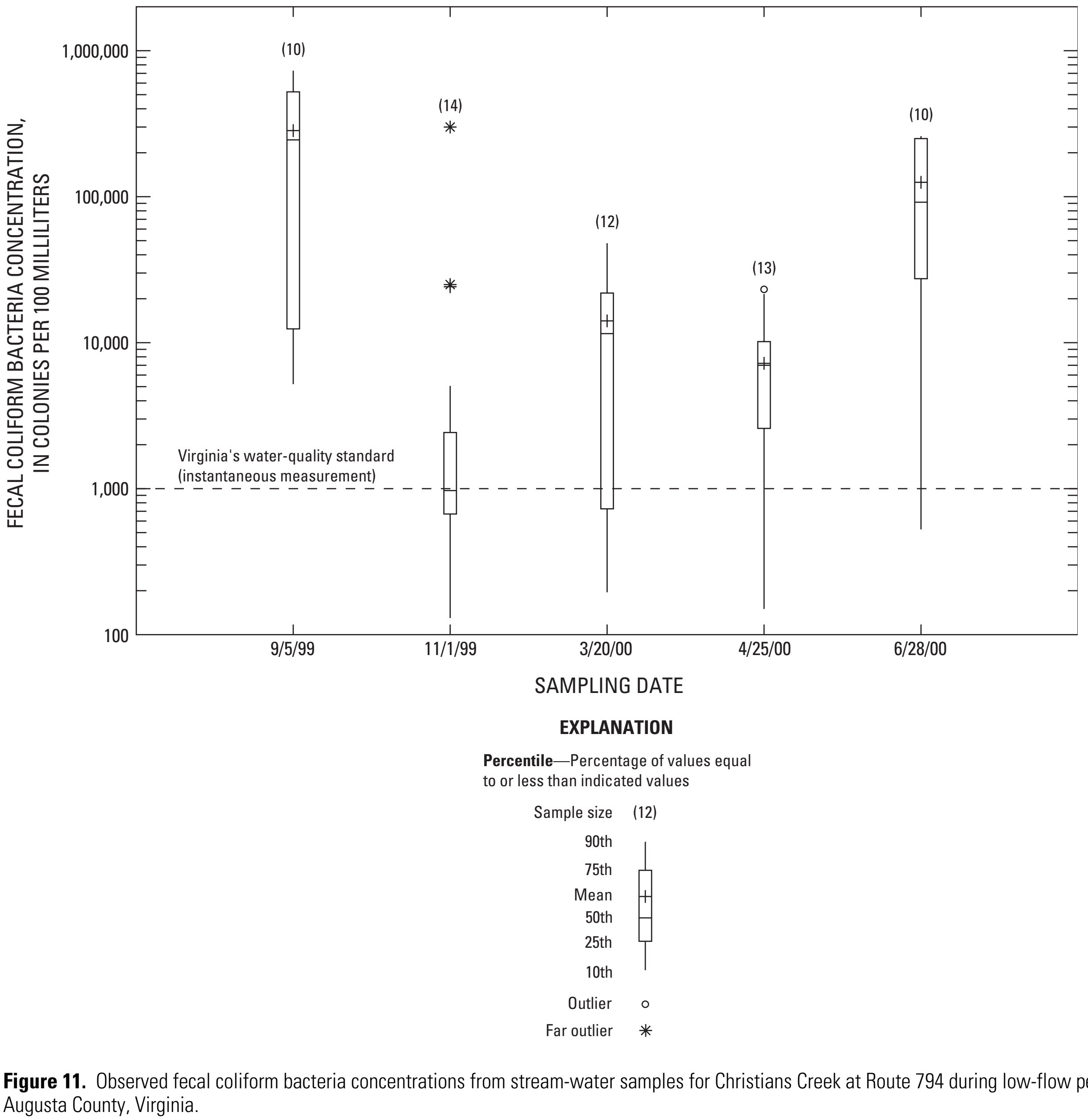


Figure 11. Observed fecal coliform bacteria concentrations from stream-water samples for Christians Creek at Route 794 during low-flow periods, Augusta County, Virginia.

Table 19. Fecal coliform bacteria concentrations for water-quality samples collected by the U. S. Geological Survey during low-flow and stormflow conditions at Route 794 (01624800) and at five other sites along the continuum of Christians Creek, Augusta County, Virginia

Station number[1]	Station name	Latitude Longitude	Number of samples	Fecal coliform bacteria concentration, in colonies per 100 milliliters			
				Minimum	Maximum	Mean	Median
Low-flow samples							
01624800	Route 794	38°07′42″ 78°59′41″	104	29	43,000	3,190	1,010
Stormflow samples							
01624800	Route 794	38°07′42″ 78°59′41″	66	130	730,000	78,216	7,611
Continuum samples							
01624615	Route 693	38°02′08″ 79°11′56″	3	5	71	28	7
01624620	Route 604	38°01′08″ 79°10′05″	3	87	1,500	629	300
01624660	Route 340	38°02′38″ 79°05′17″	3	230	3,800	2,010	2,000
01624700	Route 635	38°05′35″ 79°01′54″	3	23	6,400	2,774	1,900
01624800	Route 794	38°07′42″ 78°59′41″	3	15	1,800	868	790
01624900	Route 612	38°11′35″ 78°56′07″	3	9	830	326	140

[1]See figure 1 for location of stations

sistent with patterns observed in the fecal coliform bacteria collected by DEQ at Route 831 and Route 794; fecal coliform concentrations generally are higher upstream and lower downstream.

Bacterial Source Tracking

BST is a rapidly growing technology with various analytical techniques; the technique used depends on the study goals. In general, these techniques rely on molecular, genetics-based approaches (also known as "genetic fingerprinting") or phenotypic (relating to the physical characteristics of an organism) distinctions among the bacteria of different sources. There are three primary genetic techniques for bacterial source tracking. Ribotyping characterizes a small, specific portion of the bacteria's DNA sequence (Samadpour and Chechowitz, 1995). Pulsed-field gel electrophoresis (PFGE) is similar to ribotyping but typically is performed on the entire genome of the bacteria (Simmons and others, 1995). Polymerase chain reaction (PCR) amplifies selected DNA sequences in the bacteria's genome (Makino and others, 1999). Phenotypic techniques generally involve an antibiotic resistance analysis, in which resistance patterns for a suite of different concentrations and types of antibiotics are developed (Wiggins, 1996; Hagedorn, and others, 1999).

Although all the techniques described above are promising for identifying bacteria sources, the ribotyping technique was used to identify the sources of fecal coliform bacteria impairing Christians Creek (Hyer and Moyer, 2003). Ribotyping involves an analysis of the specific DNA sequence that codes for the production of ribosomal RNA (ribonucleic acid). Ribotyping has been demonstrated to be an effective technique for distinguishing bacteria from the feces of multiple animal species (Carson and others, 2001). This technique has been performed successfully and used to identify bacteria sources in both freshwater (Samadpour and Chechowitz, 1995) and estuarine systems (Ongerth and Samadpour, 1994). Furthermore, the technique has been used to identify the species-specific sources of bacteria contributing to impairments in both urban (Herrera Environmental Consultants, Inc., 1993) and wilderness systems (Farag and others, 2001). The broad applicability of ribotyping makes it well suited for use in this study.

The Microbial Source Tracking Laboratory at the University of Washington (UWMSTL) performed the bacterial source tracking for all samples in this study. Refer to Hyer and Moyer (2003) for specific details regarding the ribotyping technique used in Christians Creek.

The results from the BST study indicate that a diverse collection of organisms contribute to the impairment of Christians Creek (Hyer and Moyer, 2003). Hyer and Moyer (2003) identified 22 different sources of fecal coliform bacteria; the top 10 contributors identified by the ribotyping analysis are poultry, cattle, human, dog, horse, and deer, with cat, duck, goose, and raccoon considered minor sources, making up less than 5 percent of the total contributors (fig. 12).

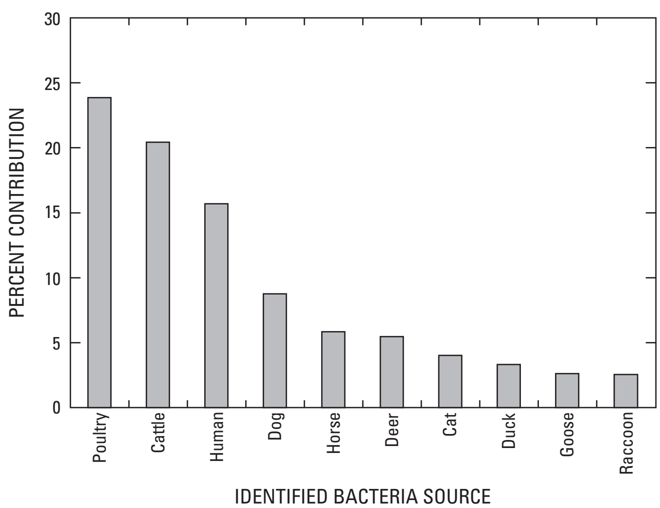


Figure 12. Distribution of the top ten contributors of fecal coliform bacteria identified by bacterial source tracking in the Christians Creek watershed, Augusta County, Virginia.

The poultry category (fig. 12) was adjusted to improve the data interpretation. The poultry category represents a combination of chicken, turkey, and other poultry sources. The ribotyping technique sometimes can be used to distinguish chickens from turkeys (and, in these cases, the two are identified separately), whereas, in other cases, an unknown isolate can be identified only as either a chicken or a turkey isolate (in this case, the isolate is labeled as poultry). Additionally, a general avian category was identified by the ribotyping analysis. The avian category represents strains of fecal coliform bacteria that can occur in multiple bird species. Whereas the poultry category was specific to chickens and turkeys, the avian category is more extensive, encompassing all birds, including chickens and turkeys. For data interpretation and watershed modeling purposes, this avian category was distributed among all the observed bird species.

Quantitatively, the avian category was assumed to be distributed proportionally, according to the occurrence of each individual bird species. For example, the poultry contribution to Christians Creek is 71 percent of all the bird species that are identified uniquely; therefore, 71 percent of the avian category was attributed to poultry. A detailed description of the manipulation of the avian category is in Hyer and Moyer (2003).

Calibration Approach

The calibrated fecal coliform model can be used to accurately simulate the range of observed fecal coliform concentration data as well as observed BST data from the Christians Creek Watershed. The simulations cover approximately a 7-year period from April 1, 1991, to September 30, 1997.

A suite of water-quality transport and storage parameters governs the simulation of fecal coliform bacteria in HSPF. As with the streamflow simulation, these parameters are categorized as fixed and adjusted. Fixed parameters can be measured or are well documented in the literature, and can be used with a high degree of confidence. The fecal coliform model parameters that were fixed (held constant) during the calibration process were the bacteria die-off rates associated with bacteria on the land surface (REMQOP) and instream (KGEN). Adjusted parameters exhibit a high degree of variability and uncertainty in the environment. Four parameters representing fecal coliform bacteria transport and storage components were adjusted to obtain a calibrated fecal coliform model for the Christians Creek watershed: fecal coliform accumulation rate (ACCUM); susceptibility of bacteria to surface runoff (WSFAC); storage of fecal coliform bacteria in interflow (IQO); and storage of fecal coliform bacteria in active ground water (AQO). The fecal coliform model was calibrated to (1) low-flow fecal coliform concentrations collected by DEQ from 1991 through 1997, (2) the range of stormflow fecal coliform concentrations collected by USGS from 1999 through 2000, and (3) BST data collected by Hyer and Moyer (2003).

The fecal coliform model first was calibrated to the data collected 1991 through 1997 by DEQ during low-flow periods. The primary sources represented in the model that contribute fecal coliform bacteria during low-flow periods are direct deposition by instream cattle, permitted point source dischargers, nonpermitted point source dischargers (straight pipes), and active ground-water discharge (AQO). The low-flow periods represented in the model were calibrated by adjusting the inputs from instream cattle and active ground-water discharge.

Next, the fecal coliform model was calibrated to data collected 1999 through 2000 by the USGS during stormflow and recession-flow periods. This step, which focused on the range of fecal coliform bacteria concentrations during peak stormflow and stormflow recession, was achieved by adjusting ACCUM and WSFAC. WSFAC was adjusted by revising the rate of surface runoff required to remove 90 percent of the surface-stored bacteria (WSQOP). The initial values of WSQOP ranged from 0.3 to 0.7 in/hr (table 20). Lower values of WSQOP result in more bacteria being washed off the land surface per unit rate of surface runoff than do higher values. Thus, decreasing WSQOP will generate increased fecal coliform concentrations during individual storm events. However, when changes to WSQOP did not produce sufficient adjustments to resulting peak fecal coliform concentrations, then ACCUM was adjusted. The post-storm fecal coliform recession rate was calibrated by adjusting the fecal coliform concentration in interflow storage (IQO). Increasing the amount of bacteria in IQO decreases the fecal coliform bacteria recession rate. The initial value of IQO was set to 1,500 col/100 mL.

Finally, the model was calibrated to BST data collected 1999 through 2000 (Hyer and Moyer, 2003). These data provide information on the sources of fecal coliform bacteria to Christians Creek and also are treated as being representative of the percent contribution by each source to the total instream fecal coliform load. Not all bacteria sources identified by means of BST were included explicitly in the model because the fecal coliform model was developed before the results of the BST study (Hyer and Moyer, 2003) were available. The minor sources identified by Hyer and Moyer (2003) not included in the model contributed a total of 2.6 percent of the *E. coli* isolates identified. However, 98.4 percent of the *E. coli* isolates identified by means of BST (including poultry, cattle, humans, dogs, horses, deer, cats, ducks, geese, raccoons, beaver, sheep, and muskrats) were represented in the model. Source-specific instream fecal coliform loads are determined by simulating each source independently. Each source-specific instream fecal coliform load is a product of bacteria transported through surface runoff, interflow, base flow, and various point sources. The sum of the source-specific fecal coliform contributions is equal to the total fecal coliform contribution used to calibrate the model to observed concentration data. The fecal coliform accumulation rate (ACCUM) is adjusted for each source represented in the model in order to calibrate the simulated source-specific instream load to observed BST data. This calibration step helps to reduce the inherent error in the calculated ACCUM value for each source. As a result, the dominant contributing sources in the watershed identified by means of BST are represented in the model.

Table 20. Initial values of WSQOP used for the various land-use types represented in the fecal coliform model for Christians Creek, Augusta County, Virginia

[WSQOP, Rate of surface runoff required to remove 90 percent of the surface-stored fecal coliform bacteria]

Land-use type	WSQOP (inch per hour)
Urban	0.5
Residential	.5
Cropland	.6
Hayland	.6
Pasture	.6
Forest	.7
Urban impervious	.3

The calibration of the fecal coliform model was evaluated through graphical comparisons and comparison of the observed historical geometric mean concentrations to the simulated geometric mean concentrations. Plots were compared of (1) simulated daily minimum and maximum fecal coliform concentrations and observed fecal coliform concentrations, and (2) simulated and observed percent contributions to instream fecal coliform load. The geometric mean is a measure of central tendency that is unbiased by extreme high and low values and is defined as

$$GM = [(a_1)...(a_n)]^{1/n} \quad (9)$$

where *GM* is the geometric mean,

$[(a_1)... (a_n)]^{1/n}$ is nth root of the product of the n quantities, $a_1, ..., a_n$.

The geometric mean of the simulated daily fecal coliform concentrations was compared to the geometric mean of the monthly samples collected by DEQ. The comparison of the simulated and observed geometric mean concentrations was done after model calibration and was not a part of the iterative calibration process.

Data Limitations

Model calibration was hindered by limitations associated with the historical fecal coliform bacteria data from DEQ. These limitations include (1) censoring of the data by upper and lower detection limits, and (2) lack of data during peak stormflow periods. DEQ collects these data to determine if a particular stream is in compliance with the State water-quality standard, not to determine the actual fecal coliform bacteria concentration. Quantitative data, however, are preferred for use during model calibration. In addition, DEQ collects these data primarily under low-flow and recession-flow conditions. The lack of data during stormflow periods limits model calibration of simulated stormflow responses. Therefore, data collected by the USGS for this study were incorporated into the model calibration process to provide information on the response of fecal coliform bacteria concentrations during stormflow periods.

The model-construction and -calibration process also was limited by the uncertainty associated with the fecal coliform accumulation rate (ACCUM) for each source. This uncertainty is linked to the four parameters used to calculate ACCUM: feces produced per day (Fprod), number of fecal coliform bacteria per gram of feces produced (FCden), population size (POPN), and habitat area (HAB). Most of this uncertainty is associated with FCden and POPN. The range of observed FCden values in previous studies (Hussong and others, 1979; Smith, 1961; Wheater and others, 1979) commonly extends over 2–5 orders of magnitude. For example, Mara and Oragui (1981) found FCden for dogs, cats, and humans ranges from 4.1 x 10^6 col/g to 4.3 x 10^9 col/g; 8.9 x 10^4 col/g to 2.6 x 10^9 col/g; and 1.3 x 10^5 col/g to 9.0 x 10^9 col/g, respectively (Mara and Oragui, 1981). Values of POPN commonly are unknown for the human, pet, and wildlife populations, and the proportion of the population that contributes to the instream fecal coliform load also is unknown. This uncertainty for each animal type is of major concern because ACCUM is the primary input parameter for the simulation of fecal coliform bacteria; ACCUM values are analogous to precipitation data in the streamflow model. As a result of the uncertainty associated with ACCUM, BST data collected by the USGS (Hyer and Moyer, 2003) were incorporated into the fecal coliform model-calibration process. By using BST data, the simulated contributions to instream fecal coliform bacteria load from each represented source were matched to the observed contributions.

REQUIREMENTS FOR THE FECAL COLIFORM TMDL

After the fecal coliform model was calibrated, the TMDL for Christians Creek was determined. The TMDL is defined as the sum of all waste-load allocations (WLAs) from point sources and load allocations (LAs) from nonpoint sources and natural background (equation 1). The TMDL includes a margin of safety (MOS) that explicitly accounts for uncertainties incorporated into the TMDL development process. In addition, the TMDL is set at a level that ensures that the fecal coliform loads from the point sources and nonpoint sources can be assimilated without exceeding the State water-quality standard.

Designation of Endpoint

Prior to identifying the TMDL for Christians Creek, a numeric endpoint was established by DEQ; this value is used to evaluate the attainment of acceptable water quality and represents the water-quality goal that will be targeted through load reduction strategies designated in the TMDL plan. The numeric endpoint for the Christians Creek TMDL was determined by DEQ and DCR on the basis of the State water-quality standards, which specify a maximum fecal coliform concentration of 1,000 col/100 mL at any time, or a geometric mean criterion of 200 col/100 mL for two or more samples over a 30-day period. The geometric mean criterion was used as the TMDL endpoint because continuous simulation modeling generates more data points than the minimum number of samples required for the calculation of the geometric mean.

Margin of Safety

An explicit 5-percent MOS, as required by DEQ and DCR, was incorporated into the TMDL for Christians Creek. Thus, the numeric endpoint was decreased from a 30-day geometric mean of 200 col/100 mL to 190 col/100 mL.

Scenario Development

The objective of load-reduction scenario development was to generate a series of scenarios that, if implemented, would generate water-quality conditions that meet the State standard, including the designated MOS, thus establishing the TMDL for Christians Creek. Each load-reduction scenario was simulated over the time period used for model calibration (1991–97). During scenario development, the fecal coliform load from a given source(s) was reduced iteratively until the target water-quality conditions were met. These load reduction scenarios then were provided to the State and local watershed managers, who then selected a scenario and designated it as the TMDL for Christians Creek.

Reductions from Point and Nonpoint Sources

Representation of permitted point source discharges in the Christians Creek model was modified during the fecal coliform load allocation assessment to reflect the current (post-2001) or proposed discharge rates for the various facilities in the watershed (table 21). Major modifications to the permitted point-source discharges used for model calibration were (1) discharge rate proposed for Fishersville sewage treatment plant (STP) (VA0025291) was increased from 0.7 mgd to 4.0 mgd, and (2) the Greenville STP (VA0090417) was added. The increased discharge rate from the Fishersville STP is a result of service-area expansion to include areas once serviced by the Staunton Plaza STP (VA0022306) and Brookwood Interchange STP (VA0022292), which were removed from service in July and August 2001. The Greenville STP was scheduled to begin discharging to Christians Creek by the end of 2002. Fecal coliform load reductions from permitted and general point sources are not required by DEQ as part of the TMDL development for Christians Creek because the dischargers are required to operate at or below the geometric mean water-quality standard of 200 col/100 mL.

Fecal coliform loads were reduced from nonpoint sources, including direct instream deposition and land surface runoff, which impact water quality during low-flow and stormflow periods. Direct instream deposition was reduced through source-specific reductions from instream cattle and straight pipes. The fecal coliform load associated with surface runoff was

Table 21. Current (post-2001) permitted point-source dischargers of fecal coliform bacteria in Christians Creek, Augusta County, Virginia

Permit number	Owner	Facility	Discharge rate (million gallons per day)	Fecal coliform permit limit (number of colonies per 100 milliliters)	Annual fecal coliform load (number of colonies per year)
VA0025291	Augusta County Service Authority	Fishersville Sewage-Treatment Plant	4.0	200	1.10×10^{13}
VA0090417	Augusta County Service Authority	Greenville Sewage-Treatment Plant	0.25	200	6.90×10^{11}
VA0020427	Augusta County School Board	Rivershead High School	0.016	200	4.41×10^{10}
VA0089061	Woodlawn Village L.L. Corp.	Woodlawn Village Mobile Home Park	0.015	200	4.16×10^{10}
VA0086738	Southern States Coop, Inc.	Southern States Coop	0	200	0
Total					1.18×10^{13}

reduced through source-specific reductions from the 13 sources represented in the model. As represented in the HSPF model, any source-specific fecal coliform load reduction on the land surface has a comparable reduction in both interflow and base flow. For example, a 75-percent reduction of dog-derived fecal coliform bacteria on the land surface will result in a 75-percent reduction of these bacteria in both interflow and base flow.

RESULTS FROM THE STREAMFLOW AND FECAL COLIFORM MODELS

Streamflow Model Calibration Results

The calibrated streamflow model was assessed initially by comparing simulated and observed streamflow at Route 794 against predefined criteria (table 22). Observed and simulated total annual runoff for water years 1994–97 was 57.87 and 58.25 in., respectively. The percent difference of 0.66 percent is within the designated 10-percent criterion and indicates that the simulated water budget closely approximates the observed water budget. The range of observed and simulated flows during the calibration period was evaluated by comparing the total of the highest 10-percent flows and the lowest 50-percent flows. The highest 10-percent flows category is representative of major storm events, whereas the lowest 50-percent is representative of base-flow conditions. The percent difference between the total of the highest 10-percent and lowest 50 percent simulated and observed flows was within the designated criteria of 10- and 15-percent difference. Additionally, the seasonality inherent in the observed and simulated seasonal flows was compared. Simulated total winter (January, February, and March) and spring (April, May, and June) runoff was –7.99 percent and –5.63 percent less, respectively, than the observed seasonal runoff. Simulated total summer (July, August, and September) and fall (October, November, and December) runoff were 1.61 (15.30 percent) and 1.47 in. (15.30 percent) greater than the observed summer and fall runoff, respectively.

The observed and simulated annual runoff for the calibration period ranged from 7.72 to 21.37 and 9.77 to 20.88 in., respectively (table 23). The percent difference between the simulated and observed annual runoff ranged from –15.82 to 26.55 percent. The long-term average annual runoff for Christians Creek for water years 1968–97 is 14.07 in. (White and others, 1998). Based on this long-term average, the streamflow model simulated runoff over a range of hydrologic extremes from dry (1995) to wet (1996).

Table 22. Observed and simulated runoff values for Route 794 for Christians Creek, Augusta County, Virginia, water years 1994-97

Runoff category	Observed (inches)	Simulated (inches)	Difference (percent)[1]	Criterion (percent)
Total annual runoff	57.87	58.25	0.66	10
Highest 10-percent flow[2]	24.90	24.26	-2.55	10
Lowest 50-percent flow[3]	10.08	10.94	8.48	15
Winter runoff	25.16	23.15	-7.99	15
Spring runoff	12.60	11.89	-5.63	15
Summer runoff	10.52	12.13	15.30	15
Fall runoff	9.61	11.08	15.30	15

[1]Value calculated as simulated minus observed divided by observed times 100.
[2]The sum of all streamflow values with a 10-percent chance or less of being equaled or exceeded, and converted to runoff values (indicative of stormflow conditions).
[3]The sum of all streamflow values with a 50-percent chance or greater of being equaled or exceeded, and converted to runoff values (indicative of base-flow conditions).

Table 23. Observed and simulated annual runoff, Christians Creek, Augusta County, Virginia, water years 1994-97

Water year	Observed (inches)	Simulated (inches)	Difference (percent)[1]
1994	14.76	15.79	6.98
1995	7.72	9.77	26.55
1996	21.37	20.88	-2.29
1997	14.03	11.81	-15.82
Total	57.87	58.25	0.66

[1]Value calculated as simulated minus observed divided by observed times 100.

Similar to total amount of runoff simulated, the pathways by which the streamflow model routes incoming precipitation is important. Total simulated runoff was derived from surface runoff, interflow, and base flow (table 24). Between 50.19 and 73.16 percent of the annual runoff for water years 1994–97 was derived from base flow (ground-water inputs). Rutledge and Mesko (1996) calculated a base-flow index of 72.2 percent for an adjacent watershed from streamflow data at South River near Dooms, Va. (station number 01626850), for the period 1981–90. Base-flow contribution to streamflow in Christians Creek varies seasonally from 72.92 percent in the spring to 50.95 percent in the summer and contributions from surface runoff during spring and summer range from 12.53 to 31.90 percent, respectively (table 24).

Various graphical comparisons provided information on the quality of the calibrated streamflow model. The hydrographs for water years 1994–97 show the simulated and observed streamflow response to individual precipitation events (fig. 13). These hydrographs show generally good agreement between simulated and observed daily mean streamflow values. A strong correlation was observed between simulated and observed streamflow where 89 percent of the variability in observed streamflow is explained by simulated streamflow (fig. 14). Residual plots display the measured difference between simulated and observed streamflow; no difference will generate a residual equal to zero. Residuals between simulated and observed streamflow in Christians Creek for water years 1994-97 are distributed uniformly around zero, indicating no bias in the model simulation (fig. 15). Flow-duration curves show the percentage of time a particular streamflow is equaled or exceeded and represent the combined effects of watershed characteristics such as climate, topography, and hydrogeologic conditions on the distribution of flow magnitude through time (Searcy, 1959). Flow-duration curves for simulated and observed daily flows in Christians Creek are similar over the majority of flow conditions except for the extreme low (less than 20 ft^3/s) flows (fig. 16).

Table 24. Simulated total annual and seasonal runoff, interflow and base flow for calibration period, Christians Creek, Augusta County, Virginia, water years 1994-97

Water year	Annual runoff (inches)	Surface runoff (inches)	Interflow (inches)	Base flow (inches)	Base-flow index (percent)
1994	15.79	2.58	3.60	9.35	59.21
1995	9.77	1.51	1.44	6.57	67.25
1996	20.88	6.15	3.95	10.48	50.19
1997	11.81	1.08	1.83	8.64	73.16
Total[1]	58.25	11.32	10.82	35.04	60.15

Water years 1994-1997	Total runoff (inches)	Surface runoff (inches)	Interflow (inches)	Base flow (inches)	Base-flow index (percent)
Winter	23.15	4.49	5.64	12.75	55.08
Spring	11.89	1.49	1.47	8.67	72.92
Summer	12.13	3.87	1.80	6.18	50.95
Fall	11.08	1.47	1.91	7.44	67.15
Total[1]	58.25	11.32	10.82	35.04	60.15

[1]May not add to indicated value because of rounding.

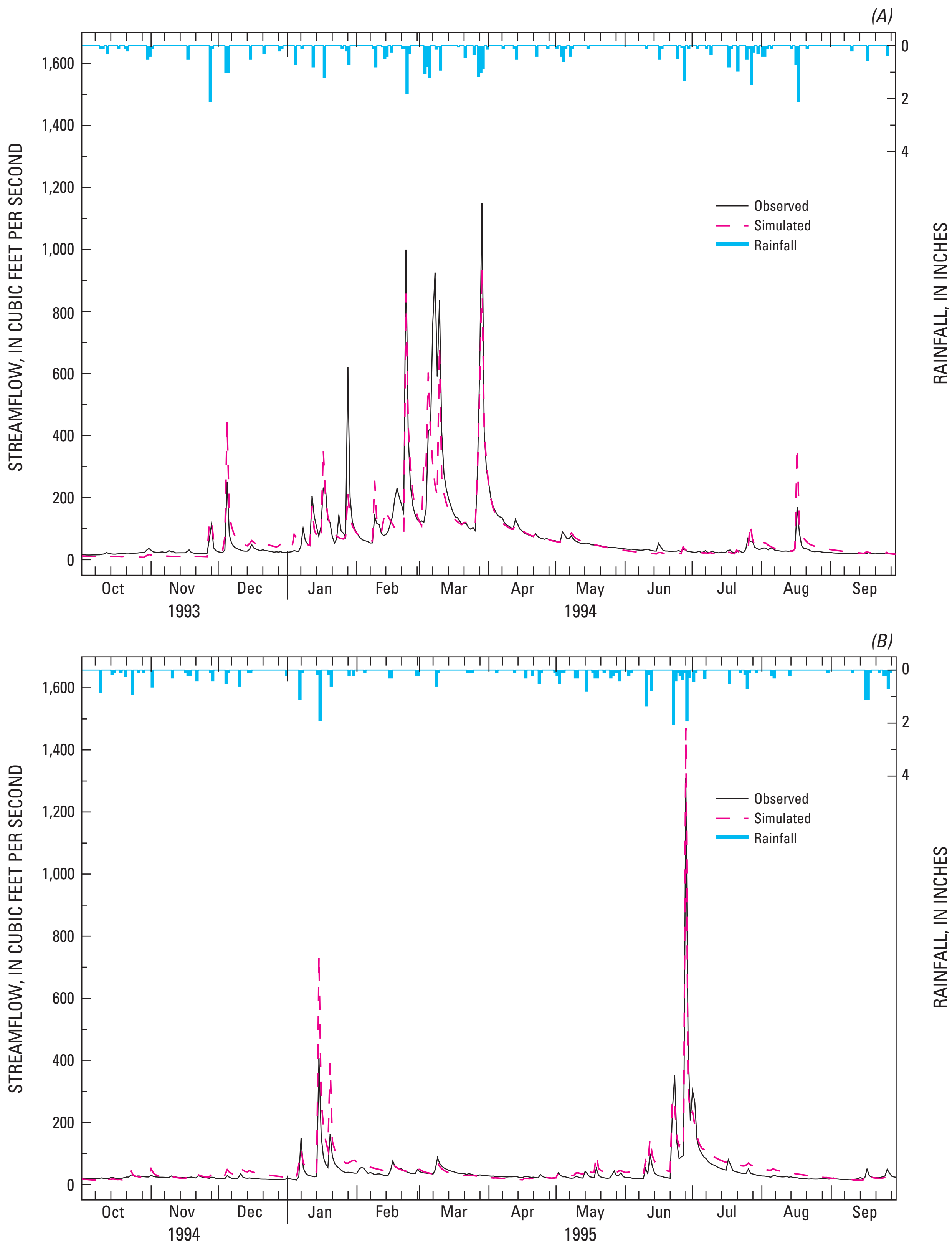


Figure 13. Daily rainfall and observed and simulated daily mean streamflows for water years 1994 *(A)*, 1995 *(B)*, 1996 *(C)*, and 1997 *(D)*, Christians Creek, Augusta County, Virginia.

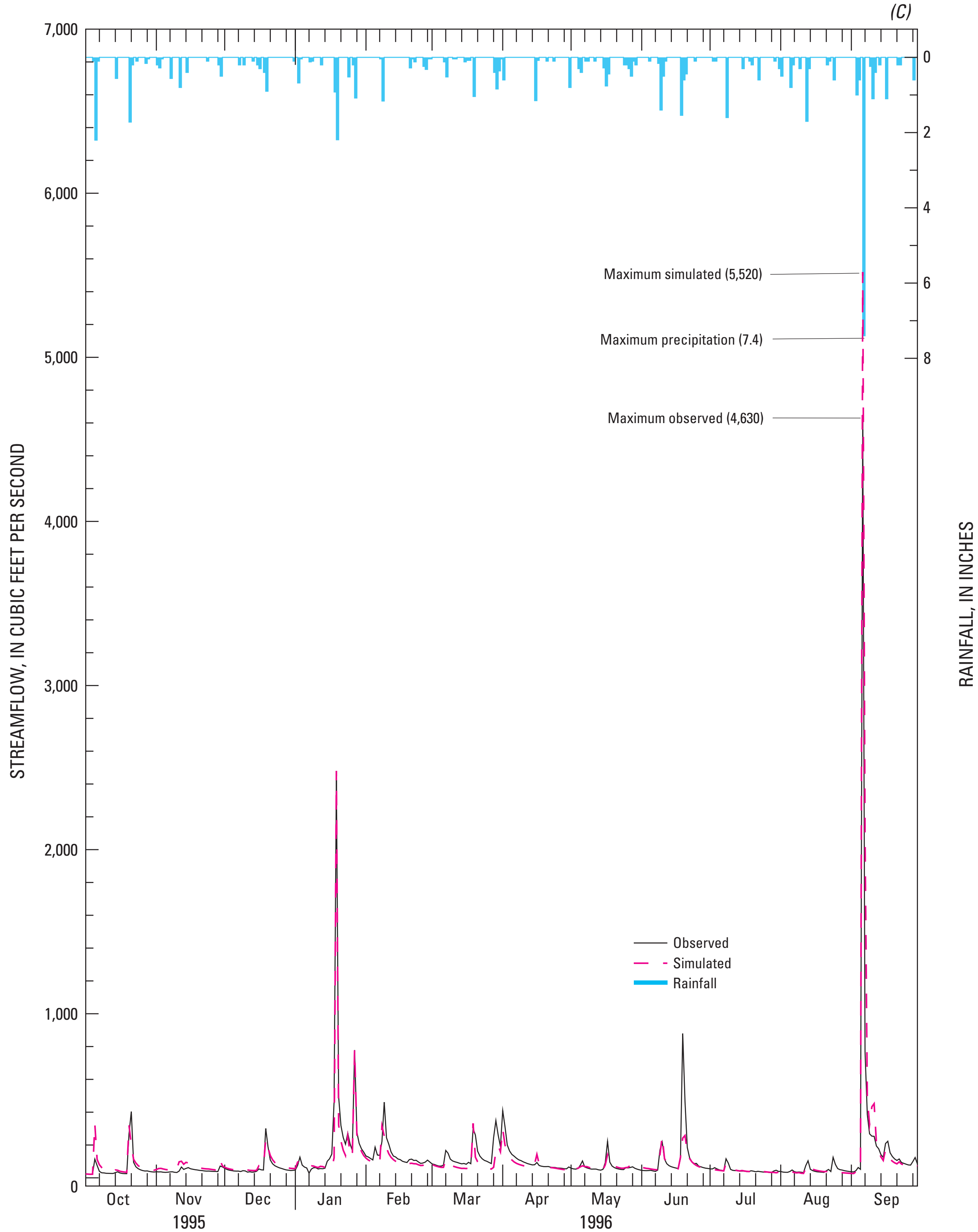


Figure 13. Daily rainfall and observed and simulated daily mean streamflows for water years 1994 *(A)*, 1995 *(B)*, 1996 *(C)*, and 1997 *(D)*, Christians Creek, Augusta County, Virginia—Continued.

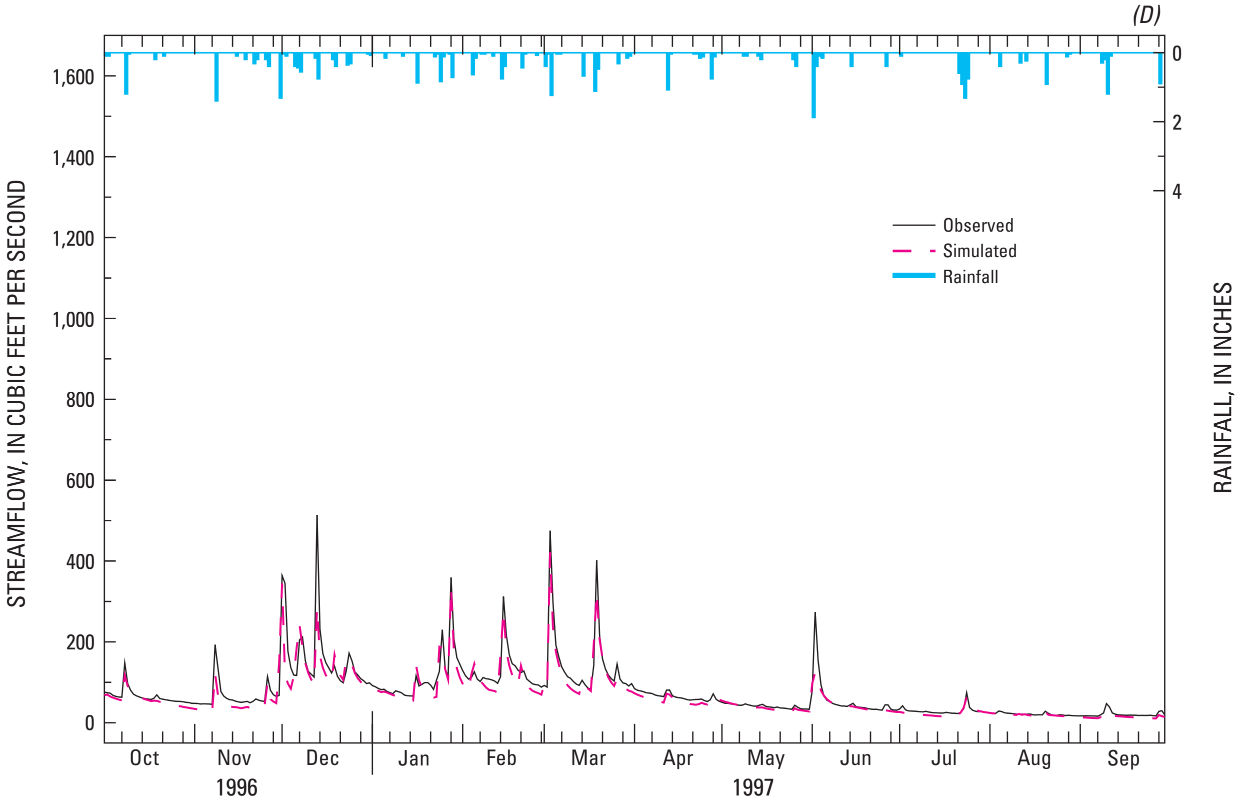


Figure 13. Daily rainfall and observed and simulated daily mean streamflows for water years 1994 *(A)*, 1995 *(B)*, 1996 *(C)*, and 1997 *(D)*, Christians Creek, Augusta County, Virginia—Continued.

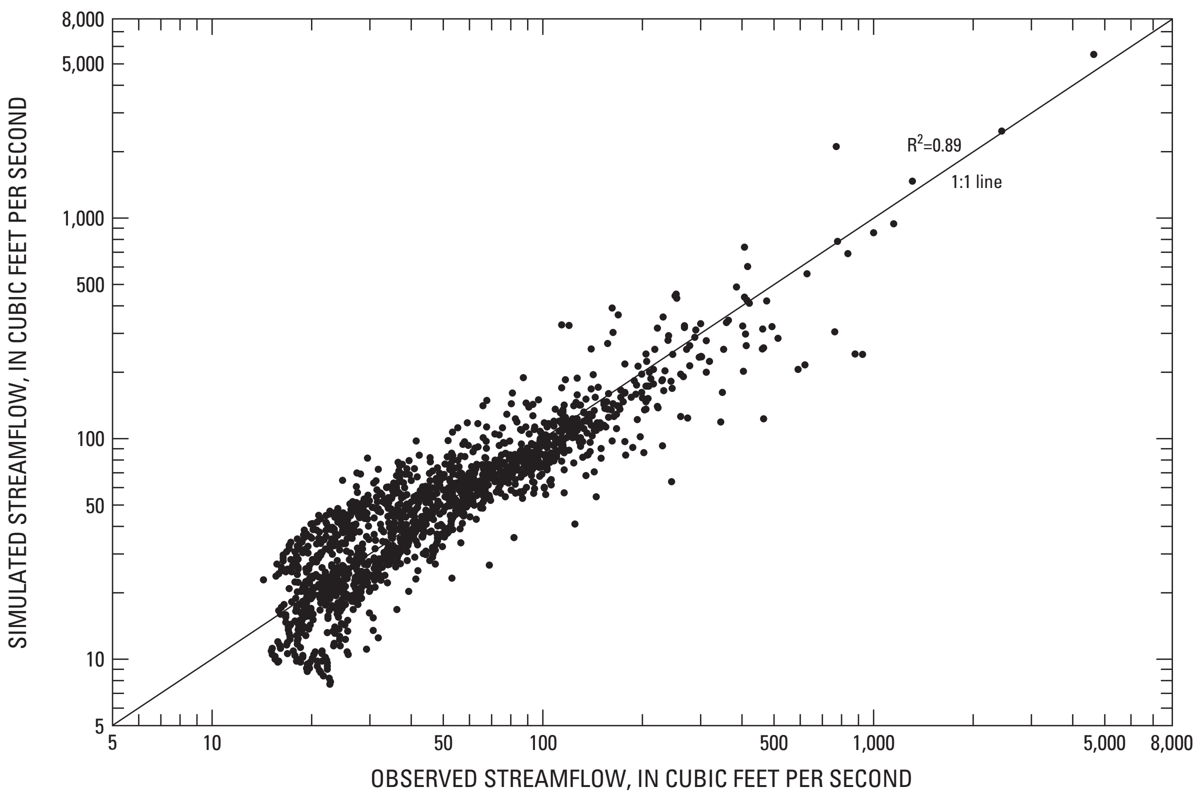


Figure 14. Simulated daily streamflow in relation to observed daily streamflow, at Christians Creek, Augusta County, Virginia, water years 1994-97.

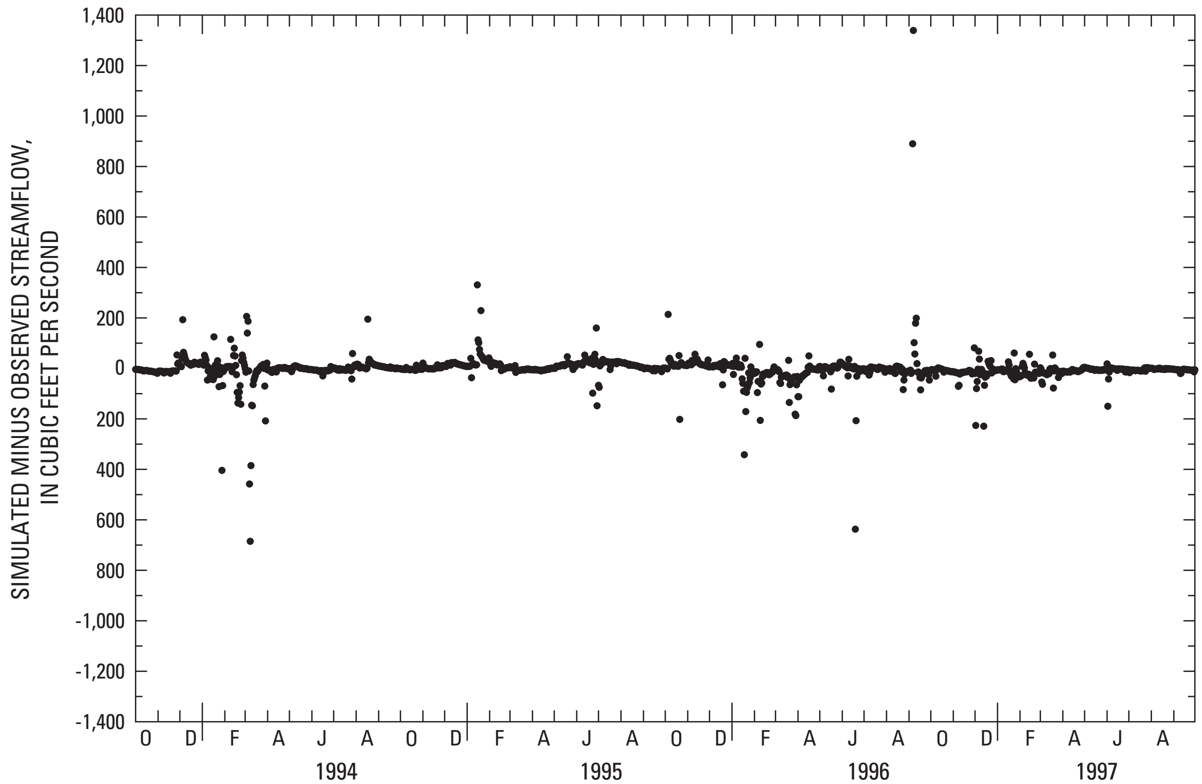


Figure 15. Residuals for simulated minus observed daily streamflow, Christians Creek, Augusta County, Virginia, water years 1994-97.

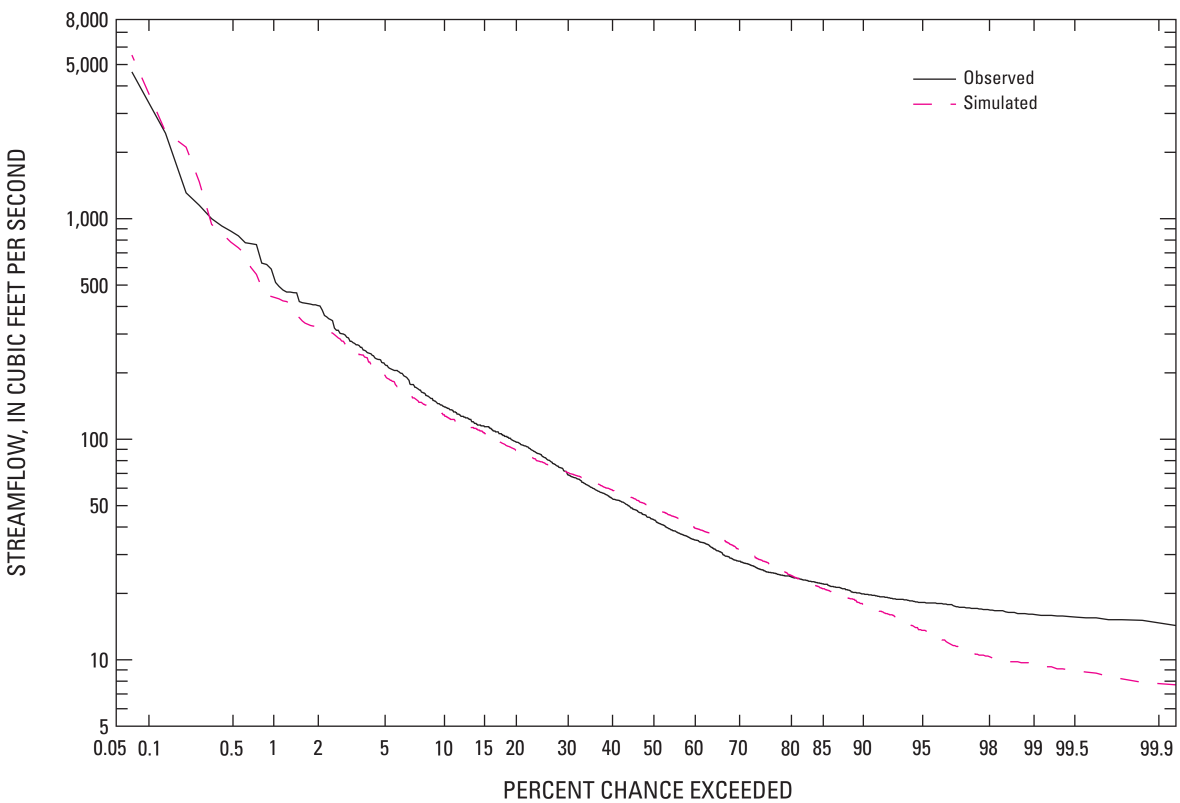


Figure 16. Flow-duration curves for observed and simulated daily mean streamflow, Christians Creek, Augusta County, Virginia, water years 1994-97

Graphical comparisons also were used to further evaluate the observed and simulated seasonal hydrologic response in Christians Creek. The distribution of simulated and observed daily flows during the winter, spring, summer, and fall months shows that simulated and observed flows for each season have similar means, medians, and variability (fig. 17). In addition, simulated flow-duration curves for winter, spring, and summer closely approximate the observed seasonal flow-duration curves (fig. 18). The simulated and observed fall flow-duration curves are similar over the majority of the flow conditions and variability increases only during the low-flow periods.

The streamflow model calibration also was evaluated using hourly simulated and observed streamflow data. This shortened time step allows for detailed evaluation of stormflow characteristics such as timing, peak flows, volume, and flow recession. For a storm event during February 23–24, 1994, simulated and observed stormflow characteristics are similar except for stormflow timing (fig. 19A). The simulated stormflow response occurs approximately 2 hours before the observed response. This time lag is present primarily because the Staunton sewage-treatment plant (SSTP) rainfall gage is approximately 6 mi. west of the streamflow gage on Christians Creek. Storm movement in the Shenandoah Valley generally is from the southwest to the northeast; therefore, rain falls at the rain gage before falling over the rest of the watershed. The simulated streamflow peak on February 24th is a result of measured rainfall occurring at the SSTP rain gage and not over the rest of the watershed.

An example of a storm event for which the stormflow response was not well simulated resulted during September 6–7, 1996 (fig. 19B). On September 6th, approximately 7.40 in. of rain fell in association with Hurricane Fran. The discrepancies in the simulated and observed stormflow characteristics can be linked to rainfall volume and intensity data and/or model calibration. Measured rainfall at the SSTP rain gage during Hurricane Fran was 7.40 in. while 2.28, 6.84, and 7.86 in. of rainfall was measured at the nearby Todd Lake, Dale Enterprise, and Sherando rain gages (operated by the National Oceanic and Atmospheric Administration), respectively. The simulated storm hydrograph is more jagged and undersimulates runoff compared to the observed hydrograph, indicating that the rainfall volume and intensity measured at SSTP is not representative of what fell in the watershed. Another explanation is that the model is not well calibrated to simulate a storm event of this magnitude.

Input-Source Error

Three factors account for many of the differences between simulated and observed streamflow. The primary factor is the quality and representativeness of the input (rainfall) data. Other factors are the occurrence of snow in the watershed and model error that results because extreme events cannot be simulated in the model.

The most important input dataset to the streamflow model is rainfall. Because of the spatial and temporal variability associated with rainfall, however, data collected at a rain gage may not always be representative of the rainfall in the surrounding areas/watershed. Additionally, 33 percent of the rainfall data at the SSTP rain gage were missing for the period 1990–97. Rainfall data from nearby gages were used to fill these missing data. In some instances during the calibration period, in addition to the examples discussed previously, rainfall data were not representative of the actual rainfall distribution over the entire watershed. For example, on August 17, 1994, the measured daily rainfall at SSTP was 2.10 in. (fig. 13A). The simulated daily mean streamflow on August 17th was 387 ft^3/s, whereas the observed daily mean streamflow was 169 ft^3/s (fig. 13A). The amount of rainfall recorded at SSTP on this date was compared with rainfall measurements of 1.24, 1.90, 2.72, 3.68, and 4.72 in. at nearby Craigsville, Dale Enterprise, Middlebrook, and Spottswood rain gages (operated by the National Oceanic and Atmospheric Administration), respectively. Because the data recorded at SSTP fell within the range of rainfall data from surrounding gages, the data value from SSTP was used during the simulation. However, the observed streamflow data indicate that less than 2.10 in. of rain fell within the Christians Creek watershed. This result is one example of model error that occurred because of input rainfall data.

Snowfall on the watershed also caused differences between simulated and observed streamflow. Snow accumulation and melt were not included in the streamflow model for Christians Creek because winter is not a critical water-quality season with respect to fecal coliform bacteria exceedances, and snowmelt is not a dominant feature of annual runoff in the watershed. During a snowfall event, the volume of water in the

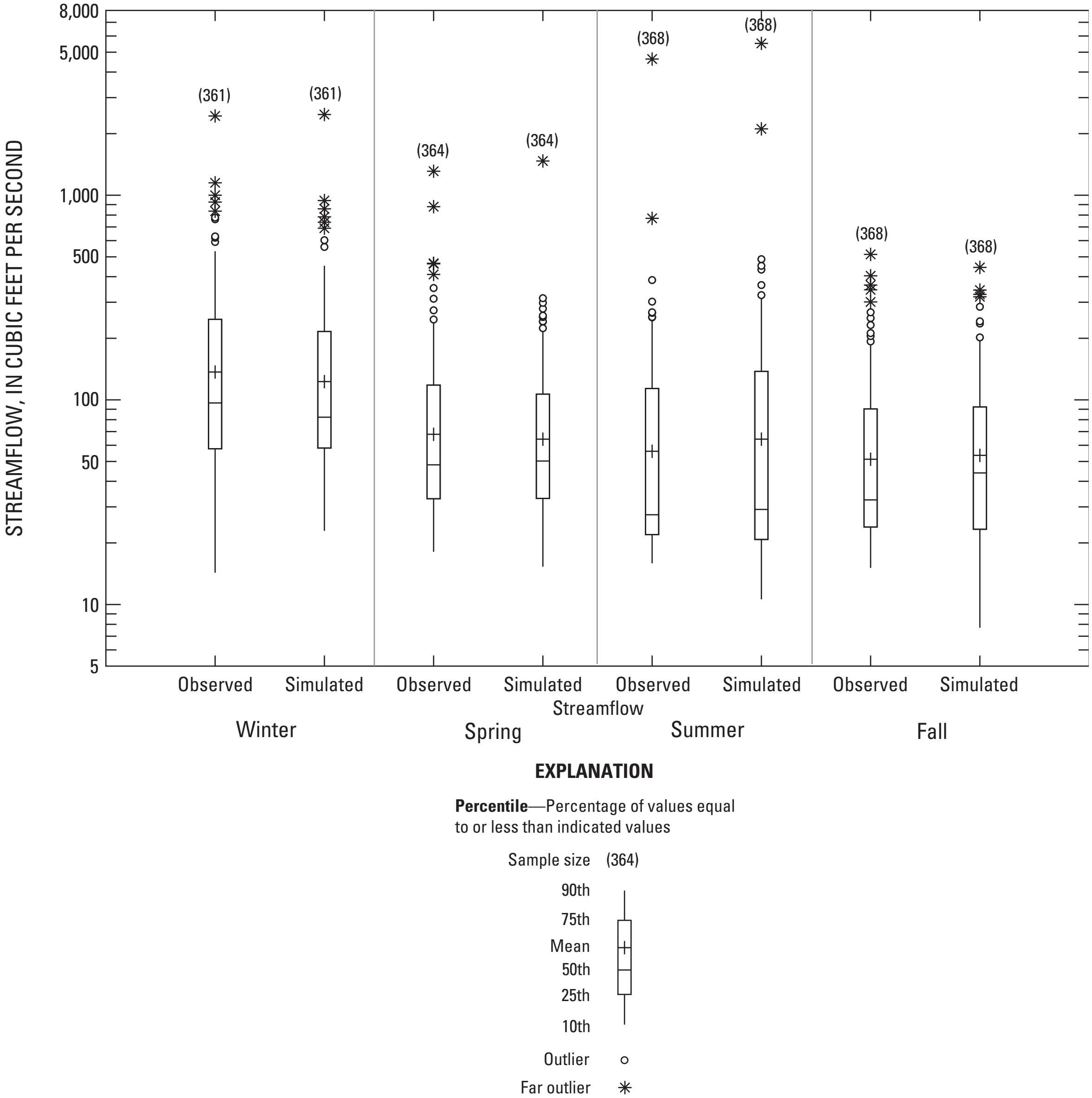
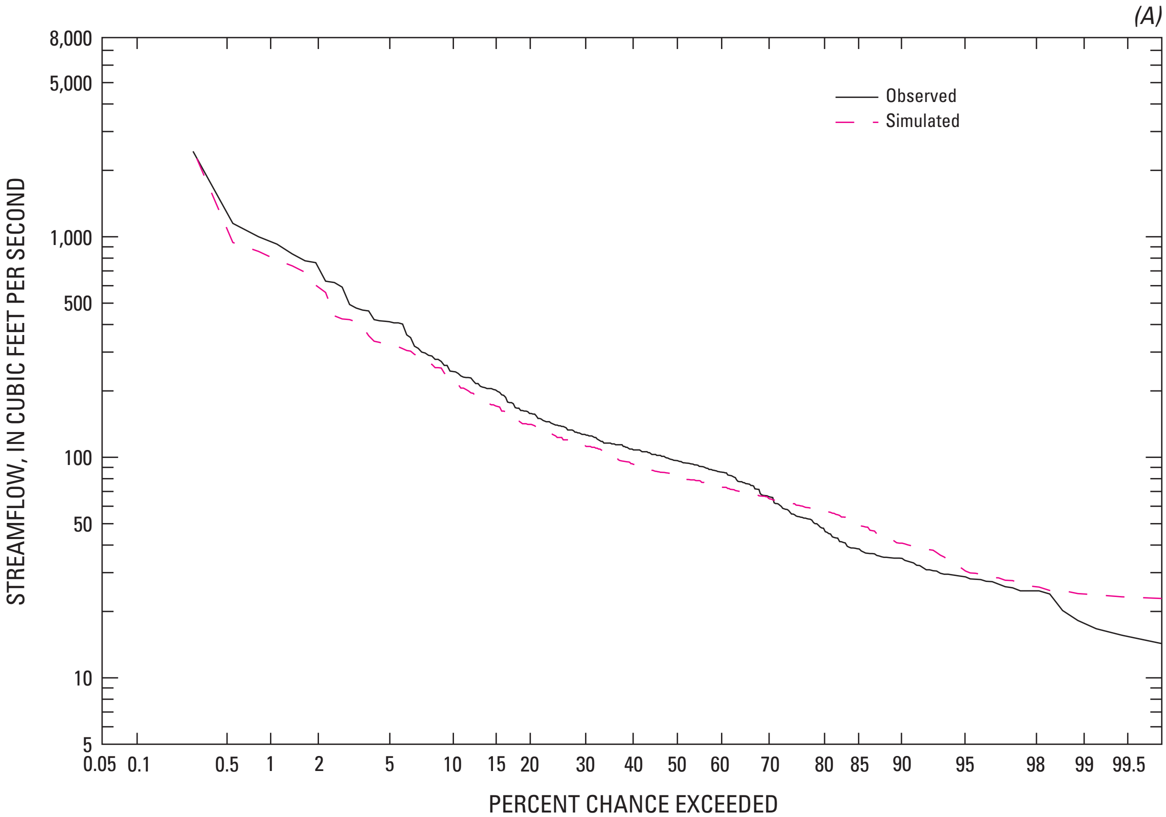


Figure 17. Observed and simulated daily streamflow (Winter, January-March; Spring, April-June; Summer, July-September; Fall, October-December), Christians Creek, Augusta County, Virginia, water years 1994-97.



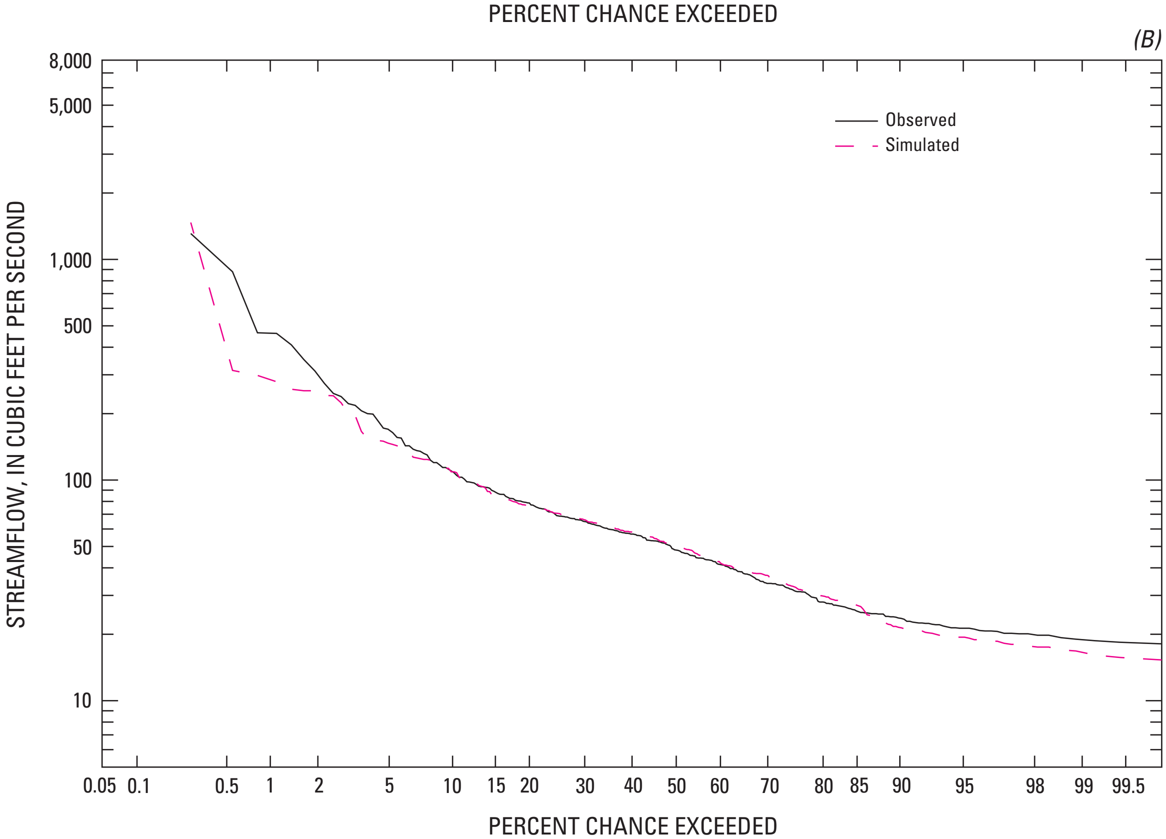
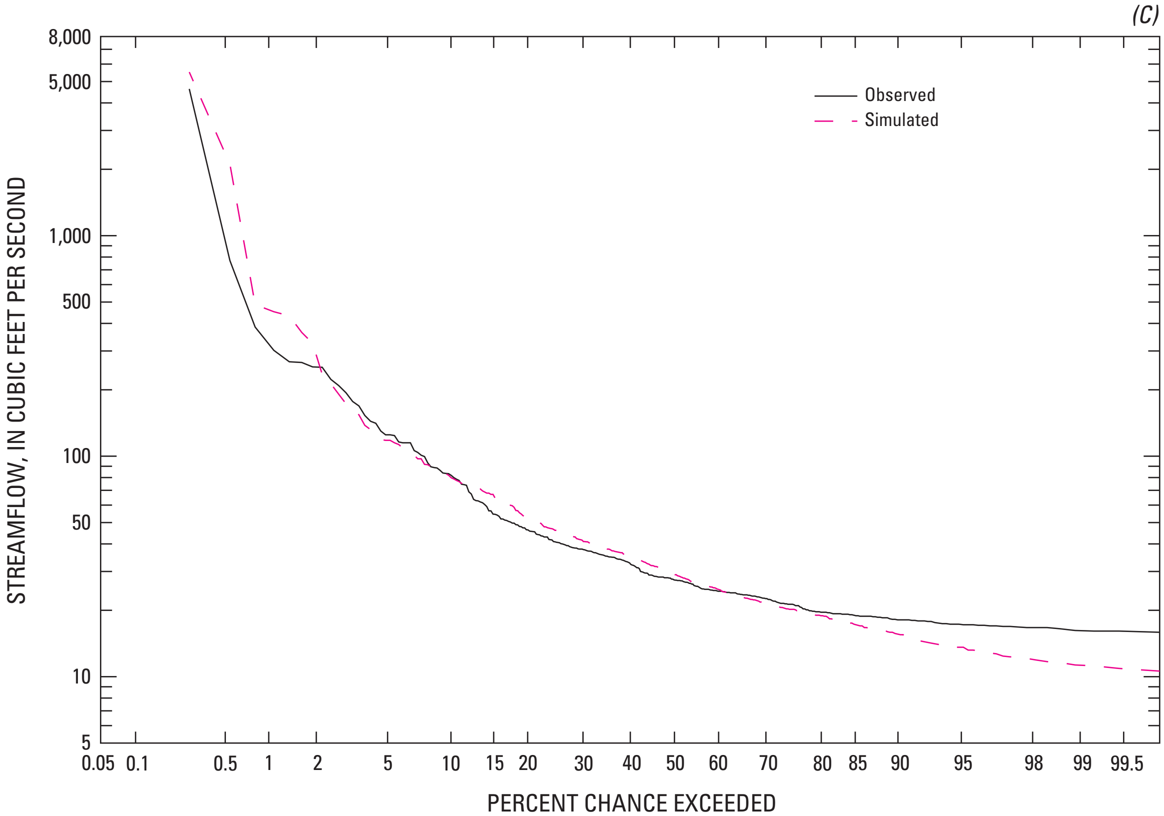


Figure 18. Seasonal flow-duration curves for observed and simulated daily mean streamflow, Winter, January-March *(A)*, Spring, April-June *(B)*, Summer, July-September *(C)*, and Fall, October-December *(D)*, in Christians Creek, Augusta County, Virginia, water years 1994-97.



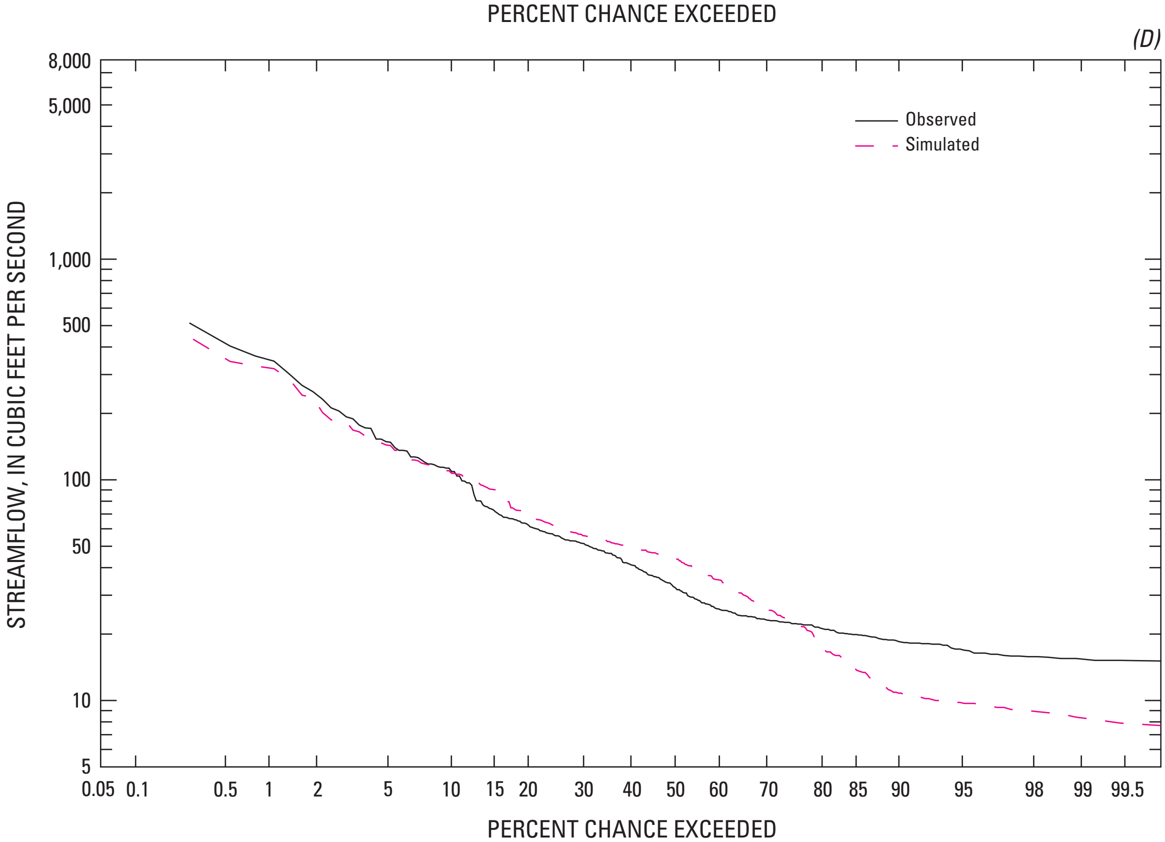


Figure 18. Seasonal flow-duration curves for observed and simulated daily mean streamflow, Winter, January-March *(A)*, Spring, April-June *(B)*, Summer, July-September *(C)*, and Fall, October-December *(D)*, in Christians Creek, Augusta County, Virginia, water years 1994-97—Continued.

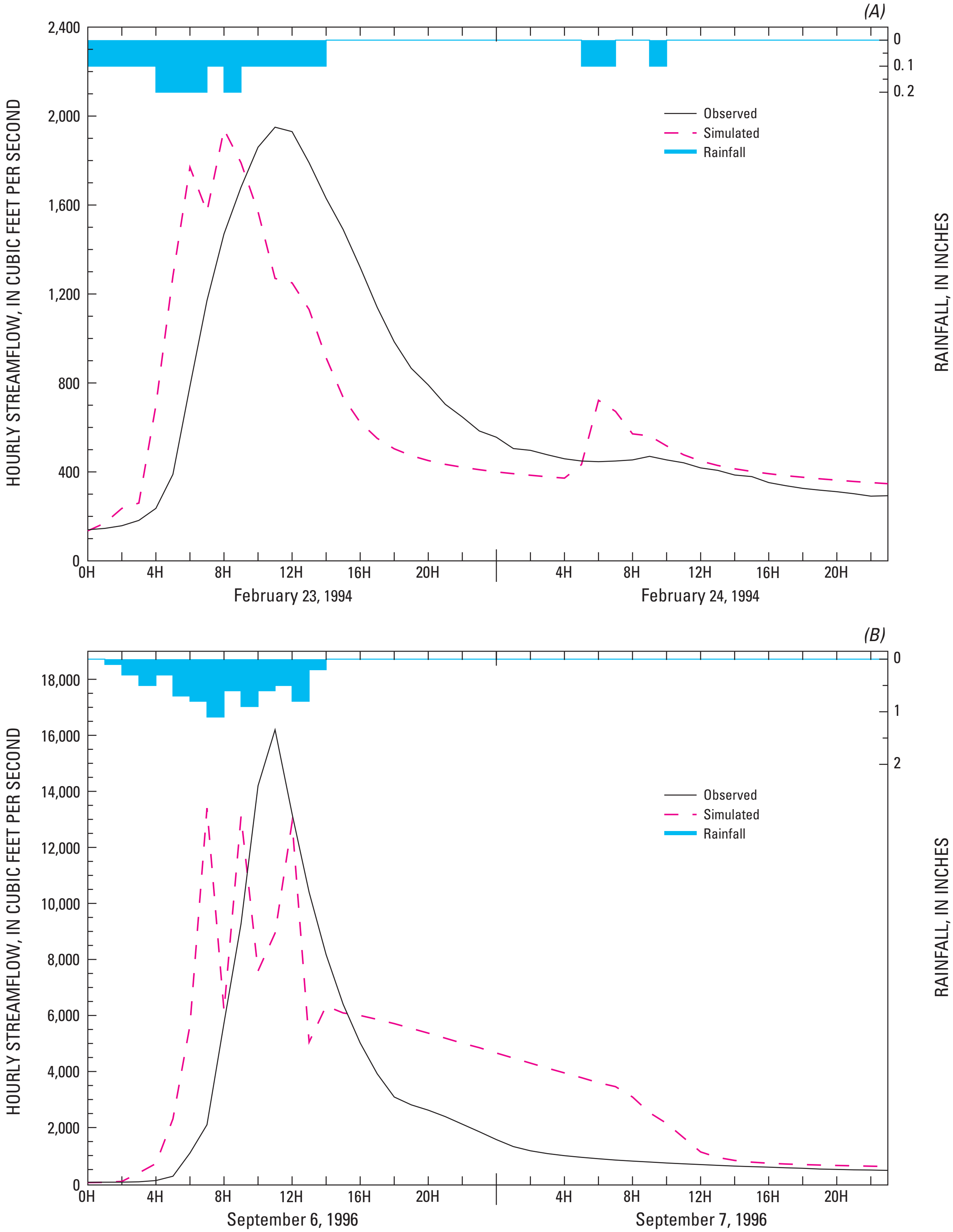


Figure 19. Hourly rainfall and observed and simulated daily mean streamflow, February 23-24, 1994 *(A)* and September 6-7, 1996 *(B)*, Christians Creek, Augusta County, Virginia.

snow typically is recorded at the rainfall gage. This recorded volume is treated as a volume of rain and used in the streamflow model. The resulting simulated streamflow response is an initial oversimulated peak followed by an extended period of undersimulated storms. The initial oversimulation is caused by the recorded volume of snow being treated like rainfall instead of the snow accumulating on the land surface. The extended period of undersimulated storms occurs because of the additional volume of water stored in the snow on the ground that is not accounted for by the model. Therefore, greater amounts of runoff per volume of incoming rain are observed than are simulated. These discrepancies resulted during the following time periods: January 14–28, 1994; March 2–15, 1994; and December 5–20, 1996 (fig. 13).

Streamflow Model Verification Results

The verification process tests the capacity of the calibrated streamflow model to simulate streamflow during a time period that was not used for model calibration and, thus, is the best test of model reliability.

Hydrologic model verification results were first assessed by comparing simulated and observed streamflow from the Route 794 stream gage for water years 1992-93 (table 25). Observed and simulated total annual runoff for water years 1992-93 was 25.96 and 25.53 in., respectively. The –1.66 percent difference is within the designated 10-percent criterion and indicates that the simulated water budget closely approximates the observed water budget. The percent difference between the total of the highest 10-percent flows was –8.24 percent. The total of the lowest 50-percent flows was 4.67 and 4.72 in. for observed and simulated flows, respectively. Simulated and observed winter (January, February, and March), spring (April, May, and June), and summer (July, August, and September) runoff were well within the designated criterion. Simulated total fall (October, November, and December) runoff was 0.68 in. (17.96 percent) greater than the observed fall runoff.

The observed and simulated annual runoff for water years 1992-93 were 10.36 and 15.60, and 10.35 and 15.18 in., respectively (table 26) which yielded –0.10 and –2.69 percent differences. The long-term average annual runoff for Christians Creek for water years 1968-97 is 14.07 in. (White and others, 1998). Based on this long-term average, the verification of the calibrated streamflow model included a dry (1992) and an average (1993) year. Total simulated runoff was derived from surface runoff, interflow, and base flow (table 27). A total of 63.18 percent of the total annual runoff for water years 1992-93 was derived from base flow (ground-water inputs), which is consistent with the findings from Rutledge and Mesko (1996) for an adjacent watershed to Christians Creek, calculated from streamflow data at South River near Dooms, Va. (station number 01626850), where the base-flow index for the period 1981–90 was 72.2 percent. Base-flow contribution to streamflow in Christians Creek varied

Table 25. Observed and simulated runoff values for Route 794, Christians Creek, Augusta County, Virginia, water years 1992-93

Runoff category	Observed (inches)	Simulated (inches)	Difference (percent)[1]	Criterion (percent)
Total annual runoff	25.96	25.53	-1.66	10
Highest 10-percent flow[2]	11.17	10.25	-8.24	10
Lowest 50-percent flow[3]	4.67	4.72	1.07	15
Winter runoff	11.33	10.76	-5.03	15
Spring runoff	9.14	8.56	-6.35	15
Summer runoff	2.24	2.38	6.25	15
Fall runoff	3.23	3.81	17.96	15

[1]Value calculated as simulated minus observed divided by observed times 100.
[2]The sum of all streamflow values with a 10-percent chance or less of being equaled or exceeded, and converted to runoff values (indicative of stormflow conditions).
[3]The sum of all streamflow values with a 50-percent chance or greater of being equaled or exceeded, and converted to runoff values (indicative of base-flow conditions).

seasonally from 86.75 percent in the summer to 50.65 percent in the winter, whereas contributions from surface runoff ranged from 4.62 percent during the summer to 21.38 percent during the winter (table 27).

Table 26. Observed and simulated annual runoff, Christians Creek, Augusta County, Virginia, water years 1992-93

Water year	Observed (inches)	Simulated (inches)	Difference (percent)[1]
1992	10.36	10.35	-0.10
1993	15.60	15.18	-2.69
Total	25.96	25.53	-1.66

[1]Value calculated as simulated minus observed divided by observed times 100.

Various graphical comparisons also were used to evaluate the results of the streamflow model verification. Hydrographs for the verification period generally show good agreement between simulated and observed daily mean values for streamflow during individual rainfall events (fig. 20). A strong correlation was observed between simulated and observed streamflow where 87 percent of the variability in observed streamflow is explained by simulated streamflow (fig. 21). This plot also shows an area where the model is underpredicting streamflow during low-flow periods, which primarily occurred in the first two months of the verification period. The residuals between simulated and observed streamflow in Christians Creek for water years 1992-1993 vary normally around zero, indicating a lack of bias in the model simulation (fig. 22). Flow duration curves for simulated and observed daily flows are similar for flows greater than 20 cfs (80 percent chance exceeded) (fig. 23). Increased separation between simulated and observed results for flows less than 20 ft^3/s. This separation can be explained by the undersimulation during the October–November 1992 time period.

Additional graphical comparisons were made to further evaluate the observed and simulated seasonal hydrologic response in Christians Creek. The distribution of simulated and observed daily flows during the winter, spring, summer, and fall months shows that simulated and observed flows for each season have similar means and medians (fig. 24). Simulated and observed streamflows for the winter and spring months exhibit nearly identical variation while simulated summer and fall streamflows had greater variability than the observed flows. Simulated and observed fall streamflows show increased variability for low flows that can be linked to the October–November 1992 time period. Flow-duration curves also illustrate how closely the model simulates the observed seasonal hydrologic response (fig. 25). Simulated flow-duration curves for winter and spring closely approximate the observed flow-duration curves. The simulated and observed flow-duration curves for summer and fall exhibit the greatest separation for flows less than 20 ft^3/s.

Table 27. Simulated total annual and seasonal runoff, surface runoff, interflow and base flow for verification period, Christians Creek, Augusta County, Virginia, water years 1992-93

Water year	Annual runoff (inches)	Surface runoff (inches)	Interflow (inches)	Base flow (inches)	Base-flow index (percent)
1992	10.35	1.62	1.64	6.83	65.99
1993	15.18	2.43	3.19	9.30	61.26
Total[1]	25.53	4.05	4.83	16.13	63.18

Water years 1992-1993	Total runoff (inches)	Surface runoff (inches)	Interflow (inches)	Base flow (inches)	Base-flow index (percent)
Winter	10.76	2.30	2.88	5.45	50.65
Spring	8.56	1.24	1.35	5.83	68.15
Summer	2.38	.11	.09	2.06	86.75
Fall	3.81	.40	.50	2.78	72.99
Total[1]	25.51	4.05	4.83	16.12	63.19

[1]May not add to indicated value because of rounding.

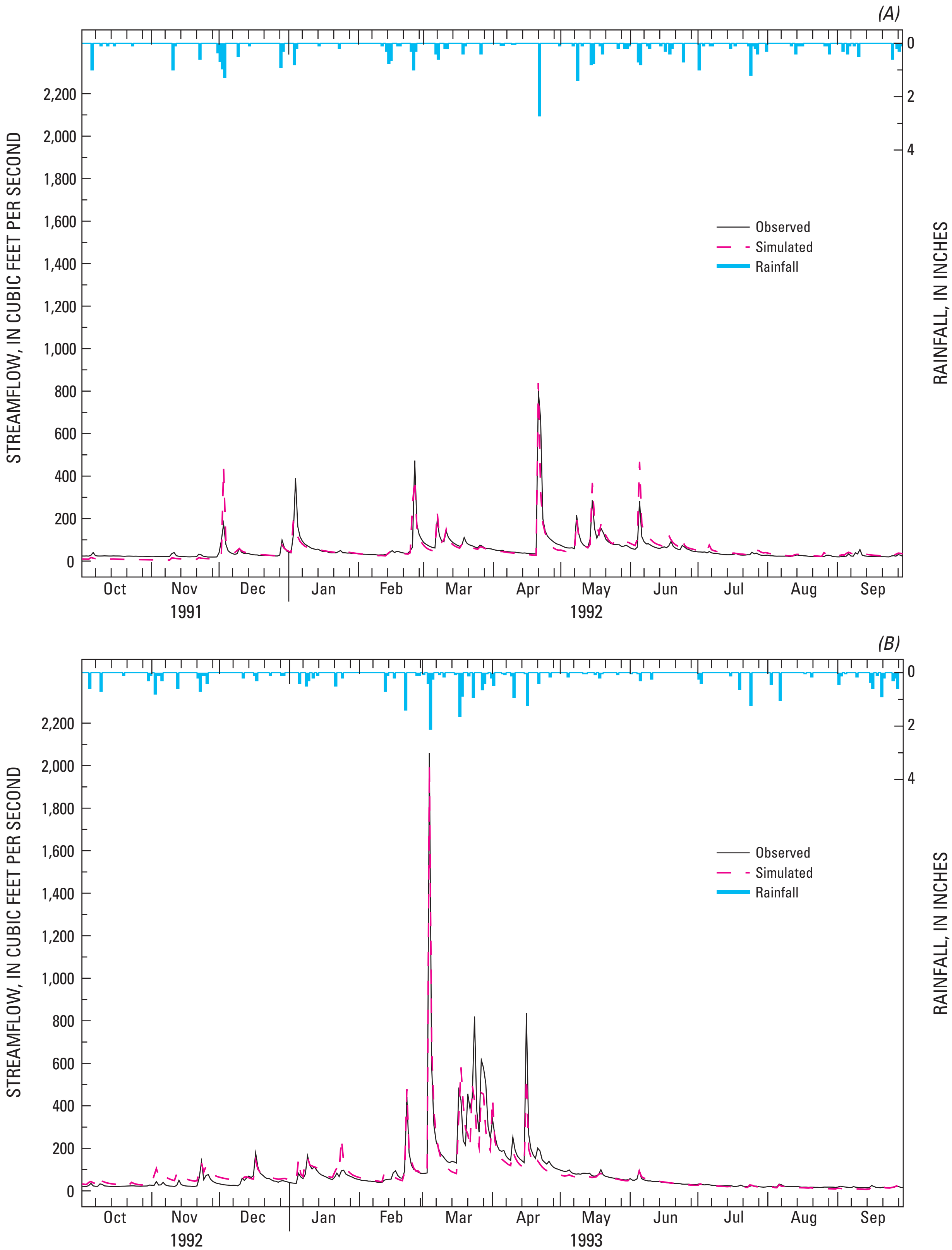


Figure 20. Daily rainfall and observed and simulated daily mean streamflow for water years 1992 *(A)*, 1993 *(B)* Christians Creek, Augusta County, Virginia.

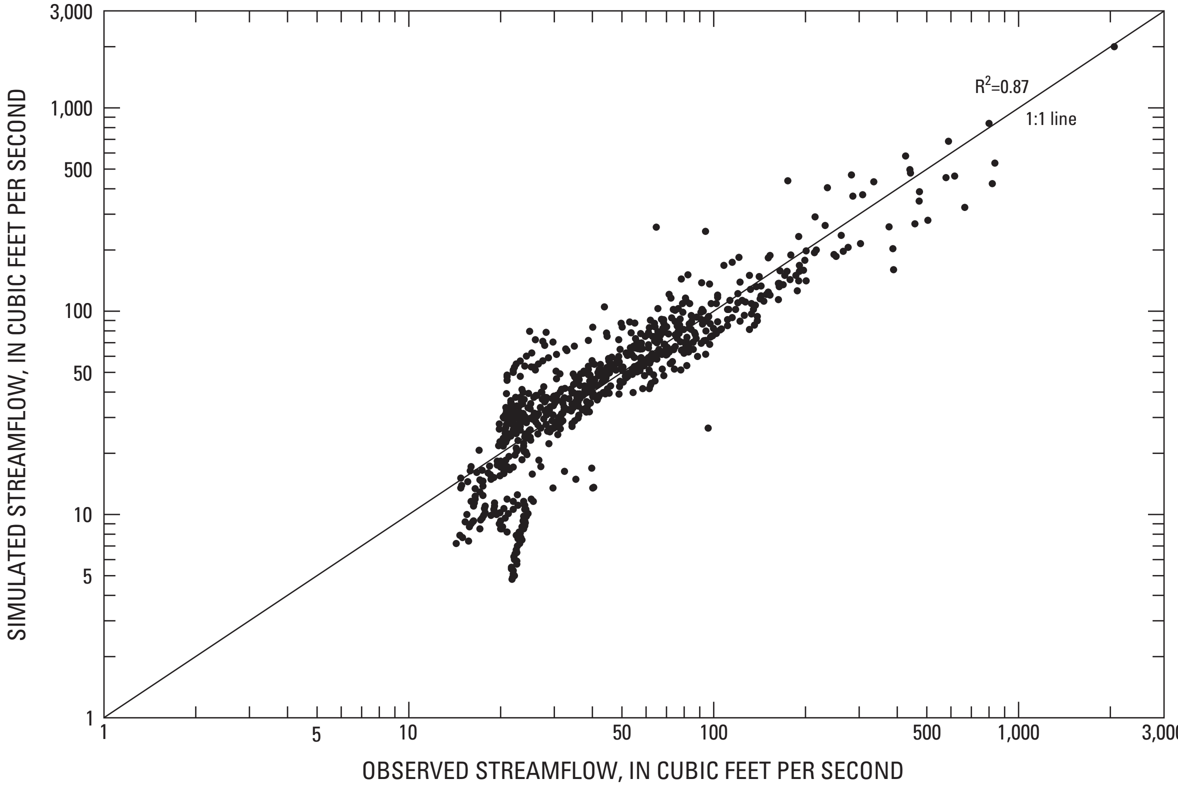


Figure 21. Simulated daily streamflow in relation to observed daily streamflow, at Christians Creek, Augusta County, Virginia, water years 1992-93.

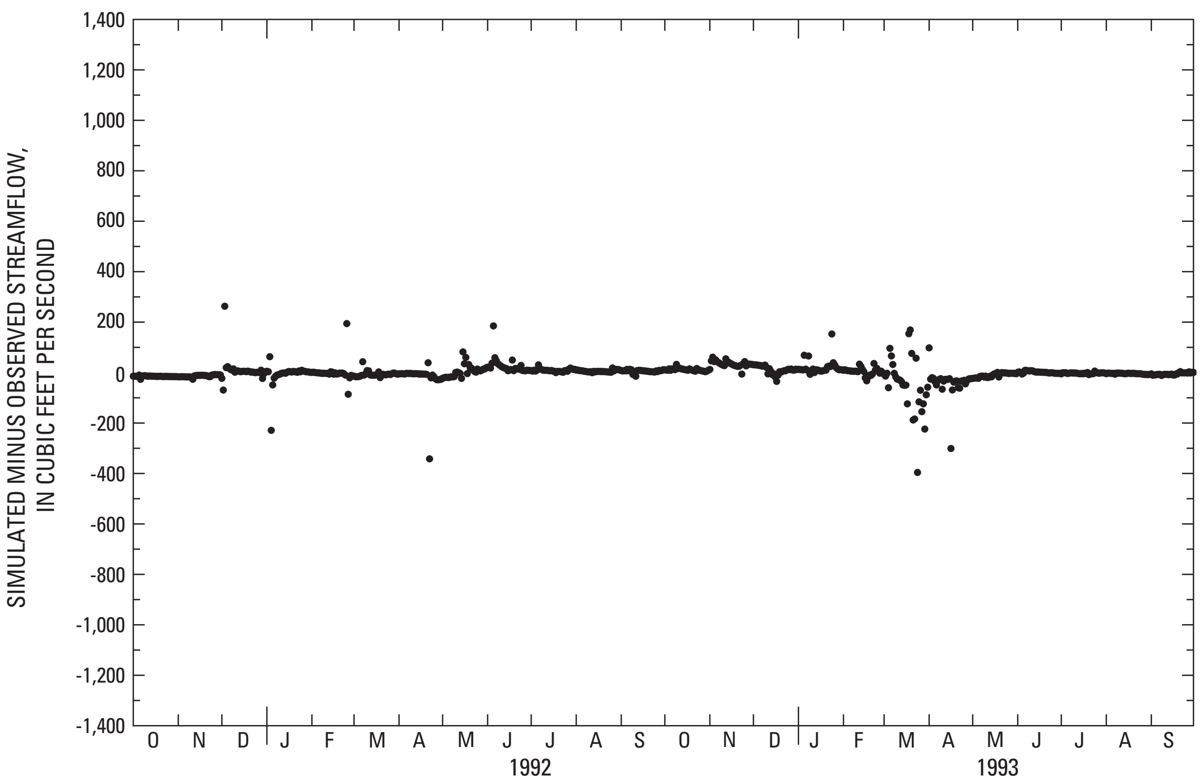


Figure 22. Residuals for simulated minus observed daily streamflow, Christians Creek, Augusta County, Virginia, water years 1992-93.

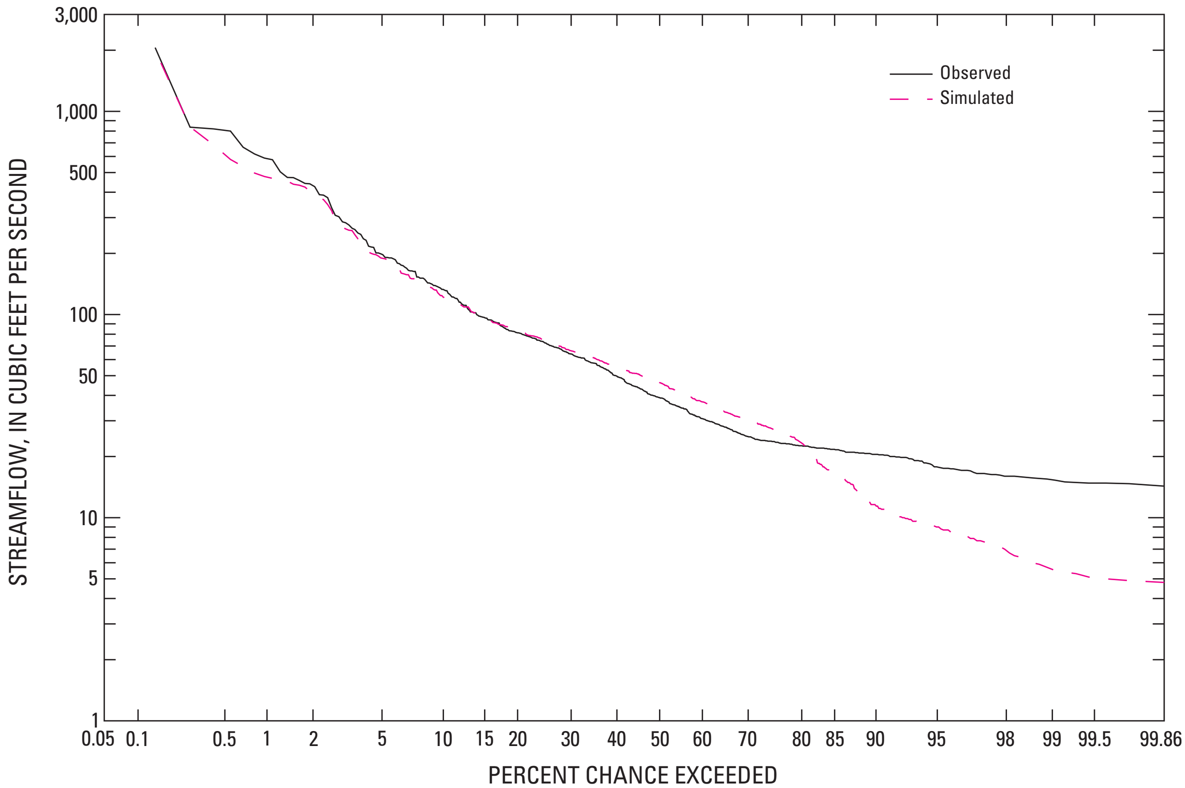


Figure 23. Flow-duration curves for observed and simulated daily mean streamflow, water years 1992-1993, Christians Creek, Augusta County, Virginia.

The streamflow model verification also was evaluated on an hourly time step. The simulated and observed stormflow responses for the April 21-22, 1992, storm event are similar with respect to storm timing, peaks, volume, and recession (fig. 26A). The simulated and observed stormflow responses did not match closely for the March 17-19, 1996, event (fig. 26B). On March 17, 1.64 in. of rain fell on top of a pre-existing 18 in. of snow and the streamflow model accounted only for the volume of rainwater, not the 18 in. of snow already on the ground. Consequently, the simulated and observed stormflow responses are different with respect to stormflow peaks and volume. The storm peak that occurred on March 18th is a result of 0.88 in. of rainfall at SSTP while a lesser amount fell over the Christians Creek watershed.

Final Streamflow Model Parameters

The results of the streamflow model calibration demonstrate its effectiveness for simulating the streamflow response in Christians Creek. Final values for the 11 hydraulic parameters used to calibrate the streamflow model and the urban effective impervious area are used in the fecal coliform model simulation (table 28).

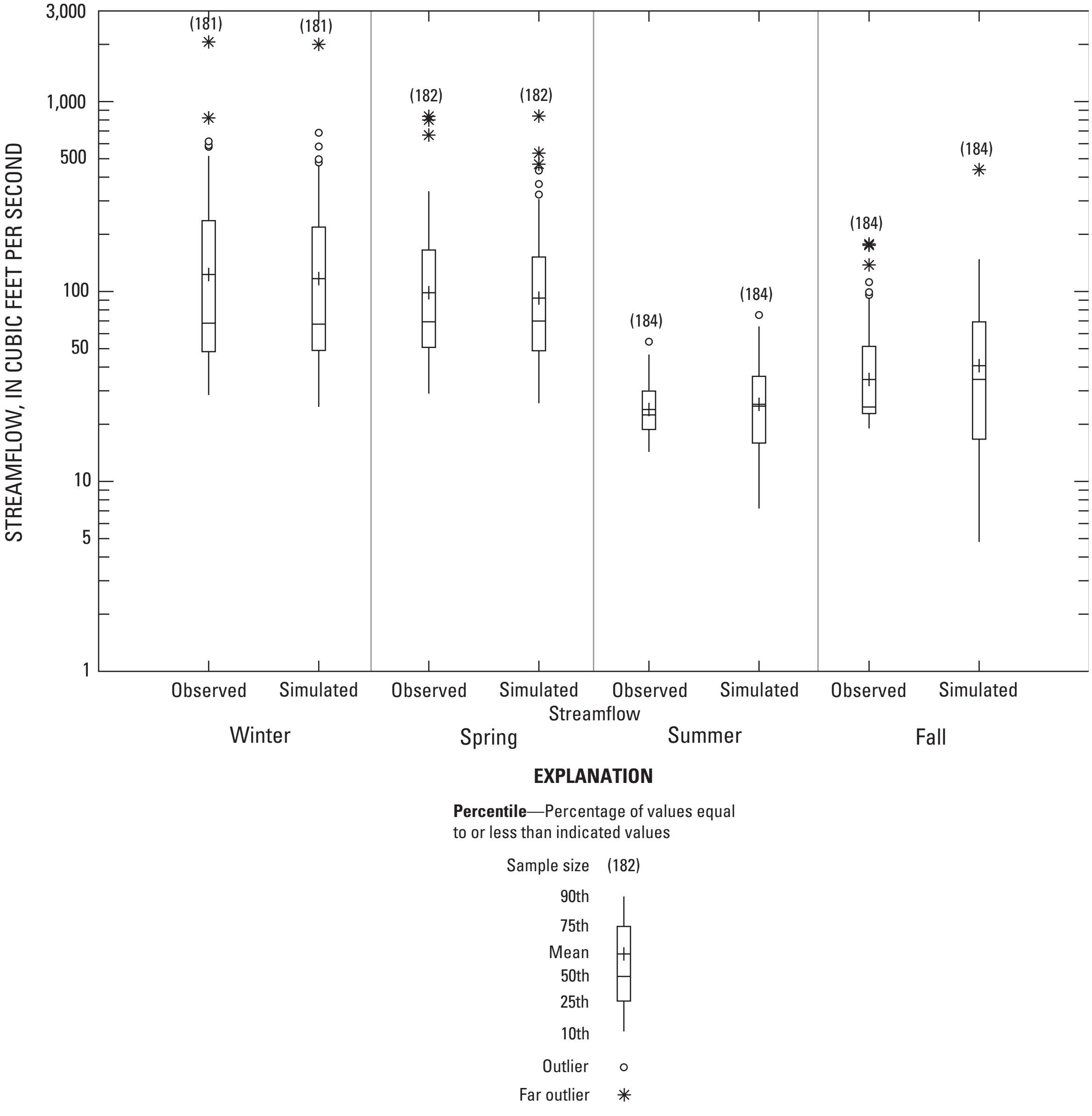
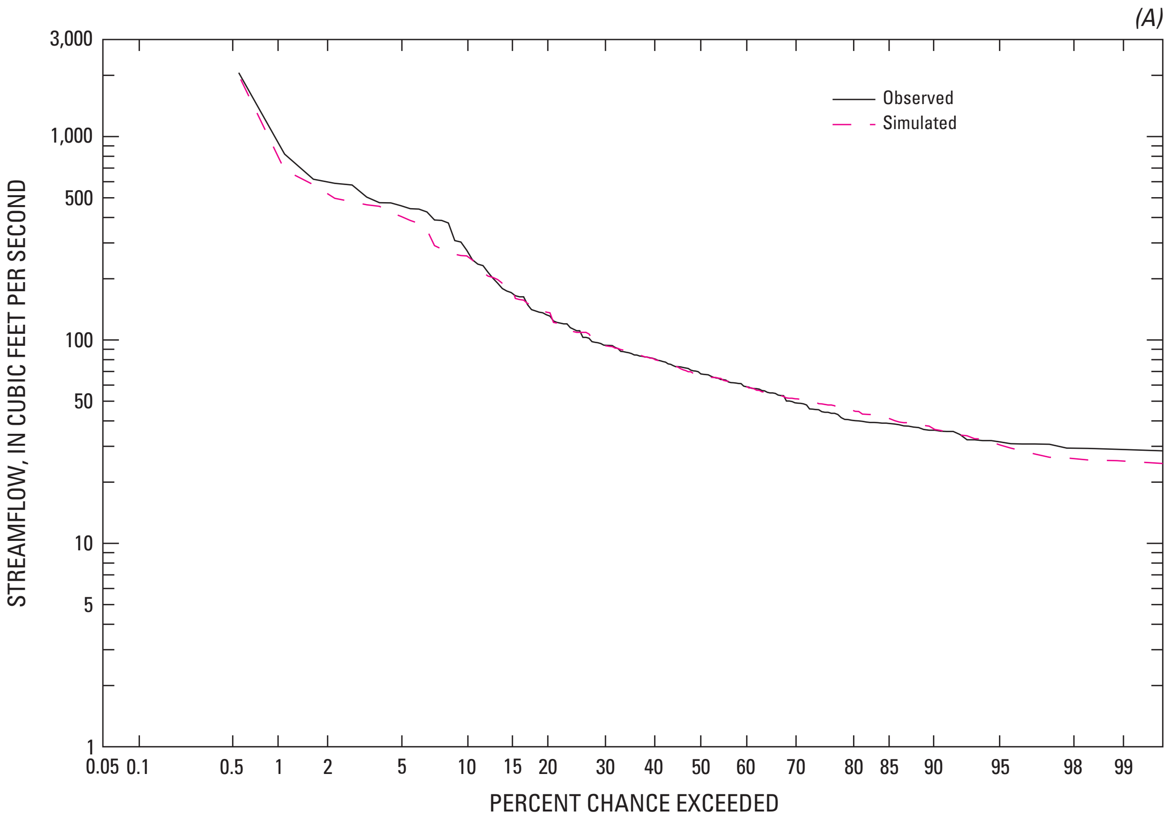


Figure 24. Observed and simulated daily streamflow (Winter, January-March; Spring, April-June; Summer, July-September; Fall, October-December), Christians Creek, Augusta County, Virginia, water years 1992-93.



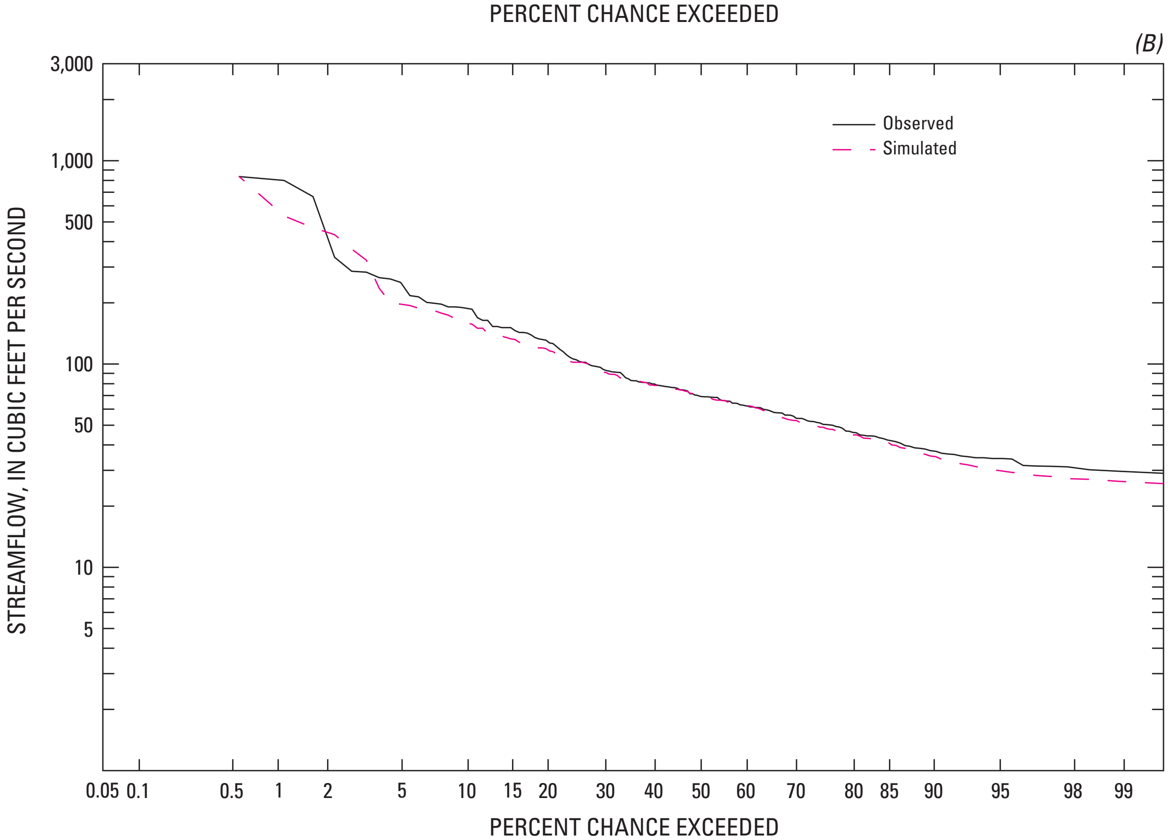
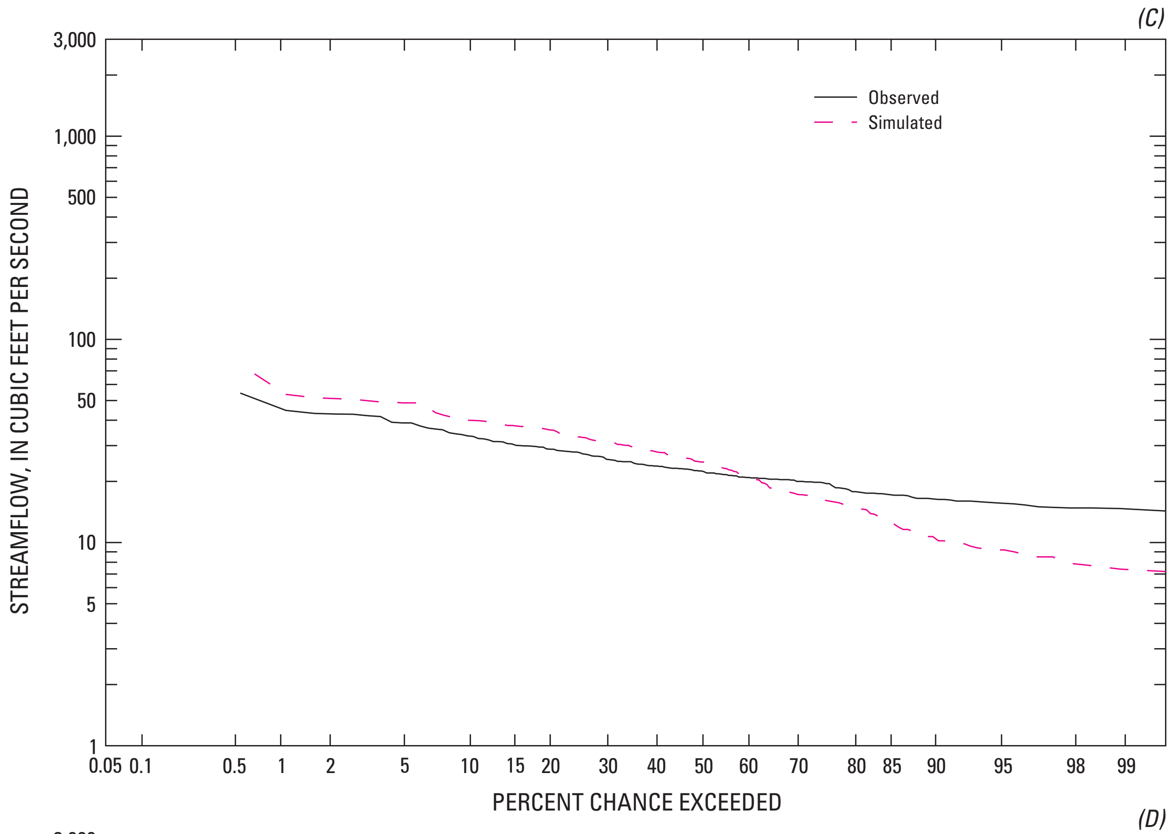


Figure 25. Seasonal flow-duration curves for observed and simulated daily mean streamflow, Winter, January-March *(A)*, Spring, April-June *(B)*, Summer, July-September *(C)*, and Fall, October-December *(D)*, in Christians Creek, Augusta County, Virginia, water years 1992-93.



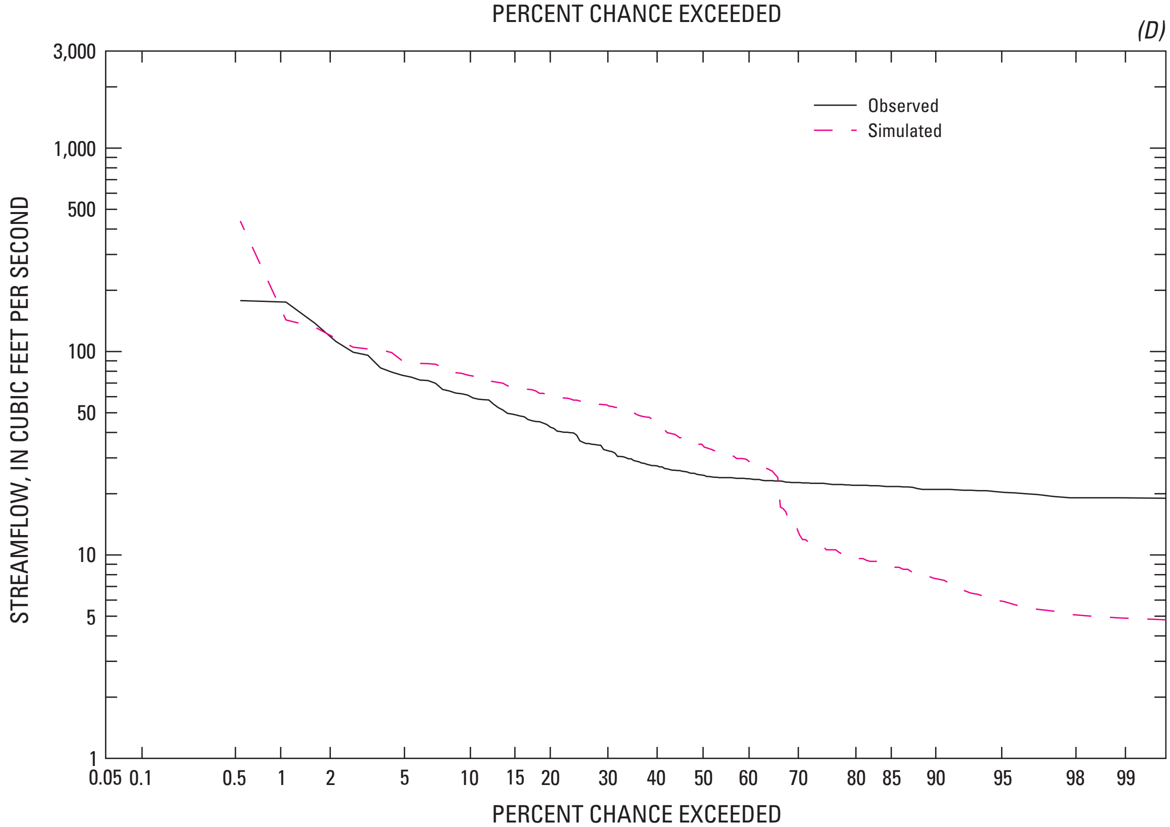


Figure 25. Seasonal flow-duration curves for observed and simulated daily mean streamflow, Winter, January-March *(A)*, Spring, April-June *(B)*, Summer, July-September *(C)*, and Fall, October-December *(D)*, in Christians Creek, Augusta County, Virginia, water years 1992-93—Continued.

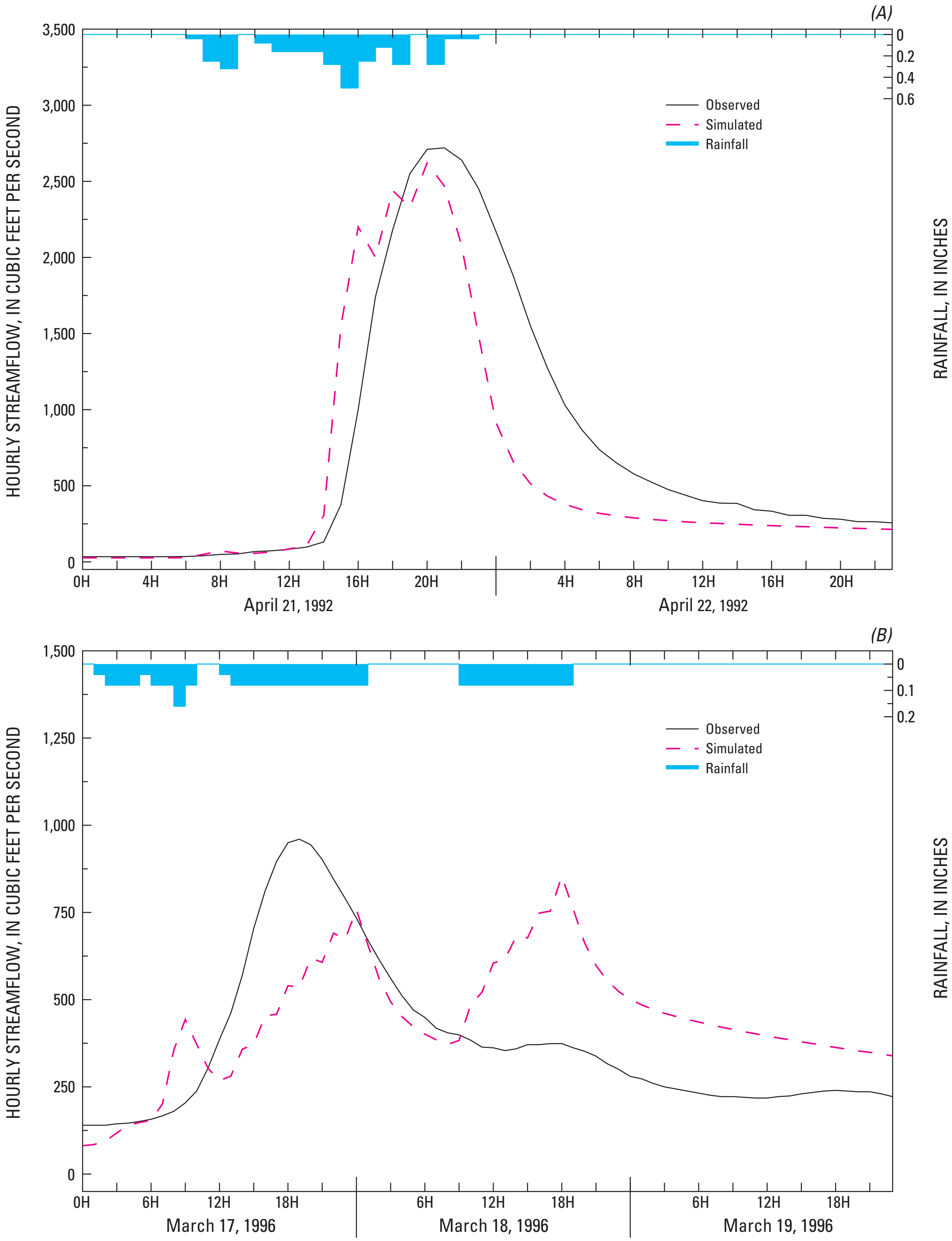


Figure 26. Hourly rainfall and observed and simulated daily mean streamflow, April 21-22, 1992 *(A)* and March 17-19, 1996 *(B)*, Christians Creek, Augusta County, Virginia.

Table 28. Final parameters and percent imperviousness used in six subwatersheds represented in the streamflow model for Christians Creek, Augusta County, Virginia

[HRU, Hydrologic Response Unit; see table 1 for definition of parameters; U, Urban; R, Residential; P, Pasture; H, Hayland; C, Cropland; F, Forest; UI, Urban impervious; PI, Pasture impervious; –, not applicable; vm, varies monthly]

HRU	Imperviousness (percent)	AGWETP	AGWRC (1 per day)	BASETP	DEEPFR	INFILT (inches per hour)	INTFW	IRC (1 per day)	KVARY (1 per inch)	LZETP	LZSN (inches)	UZSN (inches)
U	–	0.00	0.965	0.00	0.11	0.03	1.00	0.60	0.00	vm	7.00	0.70
R	–	.00	.965	.00	.11	.05	1.00	.60	.00	vm	7.00	.70
P	–	.00	.965	.00	.11	.06	1.00	.60	.00	vm	8.00	.70
H	–	.00	.965	.00	.11	.06	1.00	.60	.00	vm	8.00	vm
C	–	.00	.965	.00	.11	.06	1.00	.60	.00	vm	8.00	vm
F	–	.00	.965	.00	.11	.08	3.00	.60	.00	vm	9.00	.70
UI	6	–	–	–	–	–	–	–	–	–	–	–
PI	0	–	–	–	–	–	–	–	–	–	–	–

Fecal Coliform Model Calibration Results

The fecal coliform model is the primary tool for quantifying loads, simulating transport mechanisms, and identifying load-reduction strategies for fecal coliform bacteria in the Christians Creek watershed. Direct comparisons are made between simulated and observed fecal coliform bacteria concentrations and percent contribution from each source to instream fecal coliform bacteria load; these comparisons evaluate the effectiveness of the calibrated fecal coliform model in simulating the fate and transport of fecal coliform bacteria in the watershed.

Data from the two DEQ long-term water-quality monitoring stations, Route 831 and Route 794, were used to calibrate portions of the fecal coliform model. These DEQ data were used primarily for the calibration of fecal coliform bacteria concentrations less than 16,000 col/100 mL (mostly base-flow and recession-flow periods) because these observed data are affected by the DEQ upper detection limit for concentrations higher than 16,000 col/100 mL. USGS data collected 1999 through 2000 were used to calibrate fecal coliform bacteria concentrations greater than 16,000 colonies per 100 mL by using the range of concentrations observed during stormflow periods. The calibration results were evaluated initially by comparing graphs of simulated and observed fecal coliform concentrations. However, observed instream fecal coliform concentrations are representative only of instream conditions at the time of sample collection, whereas the fecal coliform model simulates 24 concentrations within a 1-day period. Therefore, simulated daily maximum and minimum concentrations were plotted against the observed data from Route 831 (fig. 27) and Route 794 (fig. 28). Spikes in simulated fecal coliform concentrations are the result of rainfall events where bacteria are washed off the land surface. Increases in simulated fecal coliform concentrations when spikes do not occur are the result of point source (instream cattle, straight pipes, and permitted discharges) and diffuse ground-water inputs. The capacity of the model to simulate fecal coliform concentrations during low-flow, stormflow and post-stormflow conditions was evaluated (figs. 27 and 28). In general, these conditions were simulated well in the model. The fecal coliform model had a tendency to undersimulate fecal coliform concentrations at Route 831 and oversimulate fecal coliform concentrations at Route 794. Attempts to correct Route 794 caused greater discrepancies at Route 831 and vice versa. Therefore, the resulting calibrated fecal coliform model is an average of upstream and downstream concentrations. Simulated maximum fecal coliform concentrations during storm events ranged from 1,500 to 800,000 col/100 mL. Observed maximum fecal coliform concentrations in water samples collected by the USGS at Route 794 during 1999–2000 storm events ranged from 23,000 to 730,000 col/100 mL (Hyer and Moyer, 2003). These data indicate that observed fecal

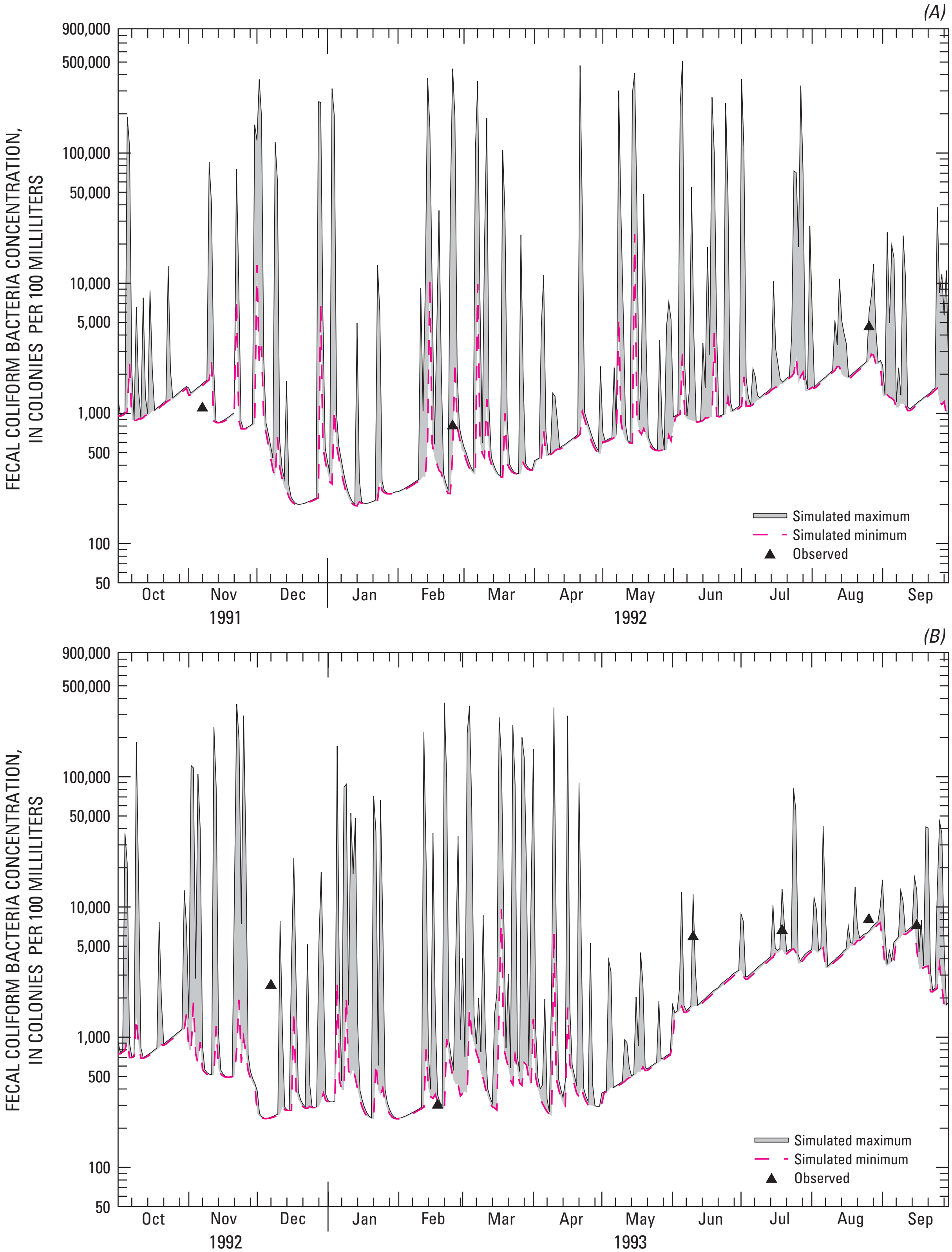
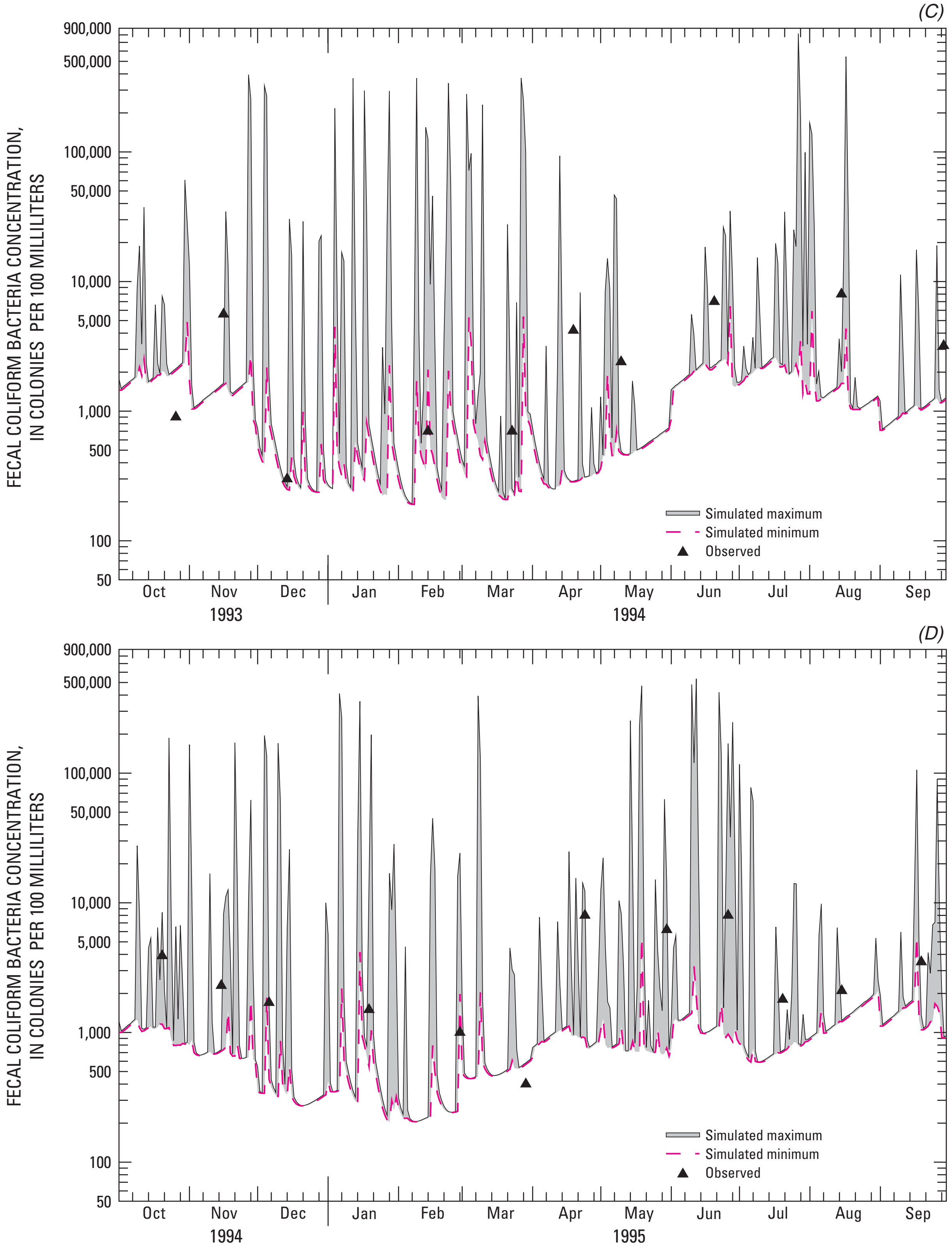


Figure 27. Simulated daily minimum and maximum concentrations, and observed instantaneous concentrations of fecal coliform bacteria at Route 831, water years 1992 *(A)*, 1993 *(B)*, 1994 *(C)*, 1995 *(D)*, 1996 *(E)*, and 1997 *(F)*, Christians Creek, Augusta County, Virginia. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)



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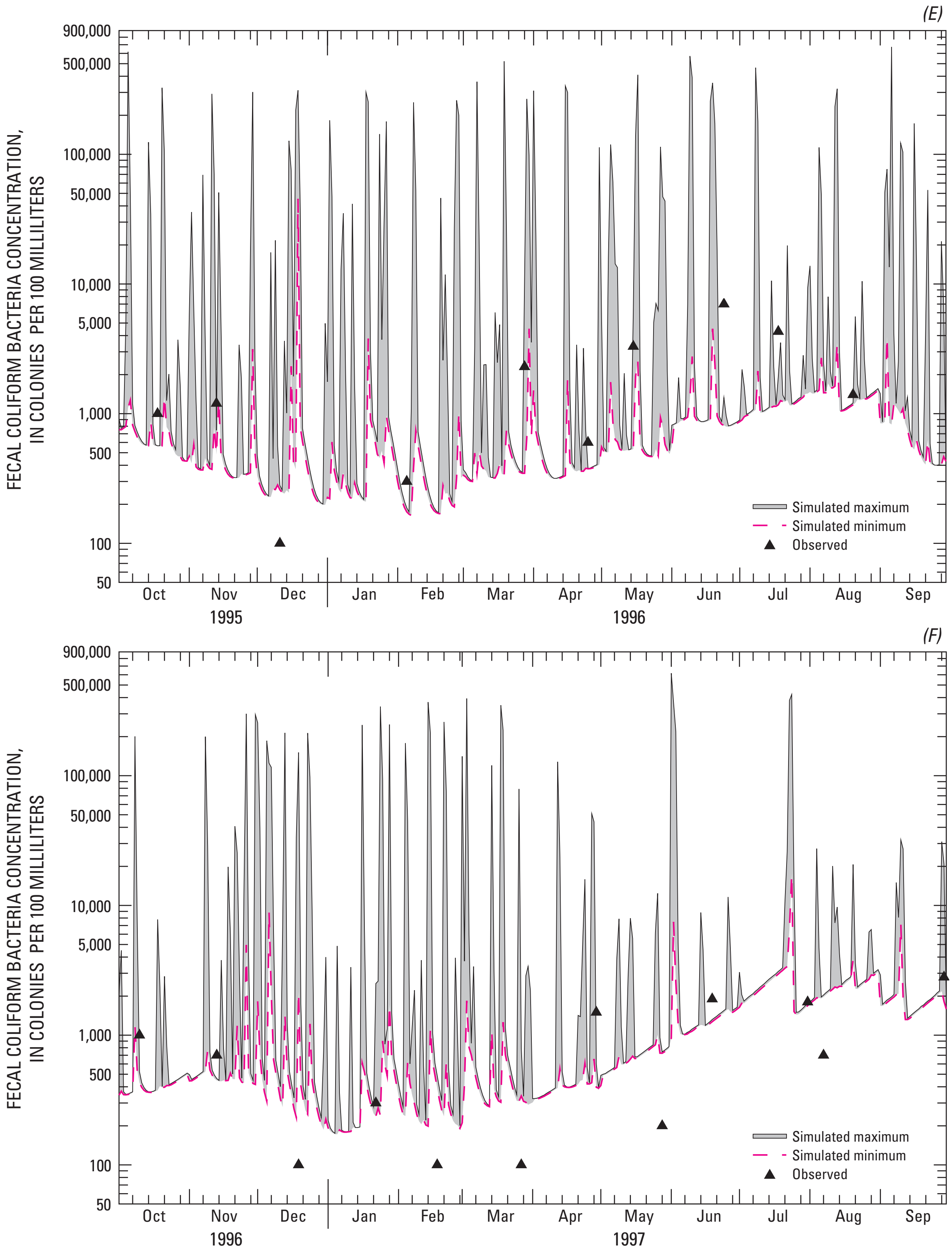


Figure 27. Simulated daily minimum and maximum concentrations, and observed instantaneous concentrations of fecal coliform bacteria at Route 831, water years 1992 *(A)*, 1993 *(B)*, 1994 *(C)*, 1995 *(D)*, 1996 *(E)*, and 1997 *(F)*, Christians Creek, Augusta County, Virginia. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)—Continued

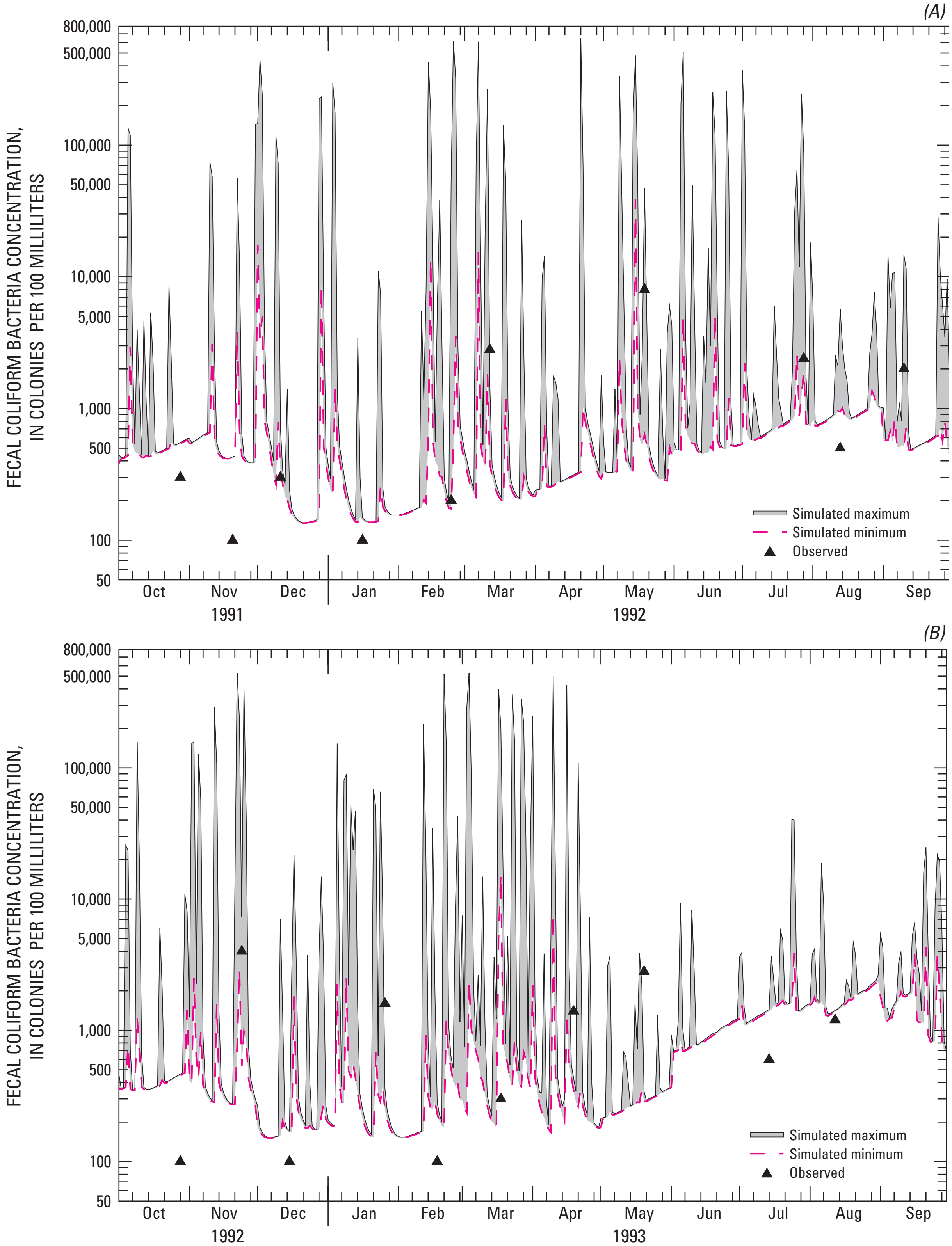
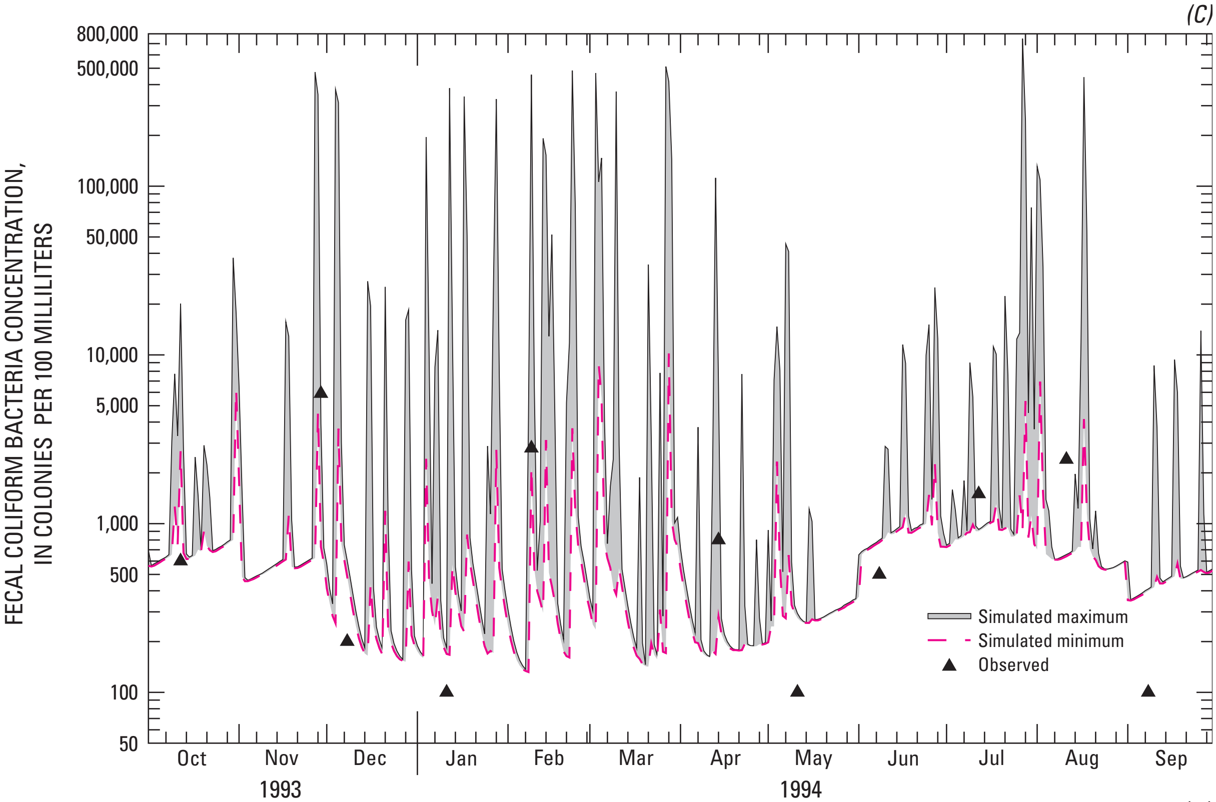


Figure 28. Simulated daily minimum and maximum concentrations, and observed instantaneous concentrations of fecal coliform bacteria at Route 794, water years 1992 *(A)*, 1993 *(B)*, 1994 *(C)*, 1995 *(D)*, 1996 *(E)*, and 1997 *(F)*, Christians Creek, Augusta County, Virginia. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)



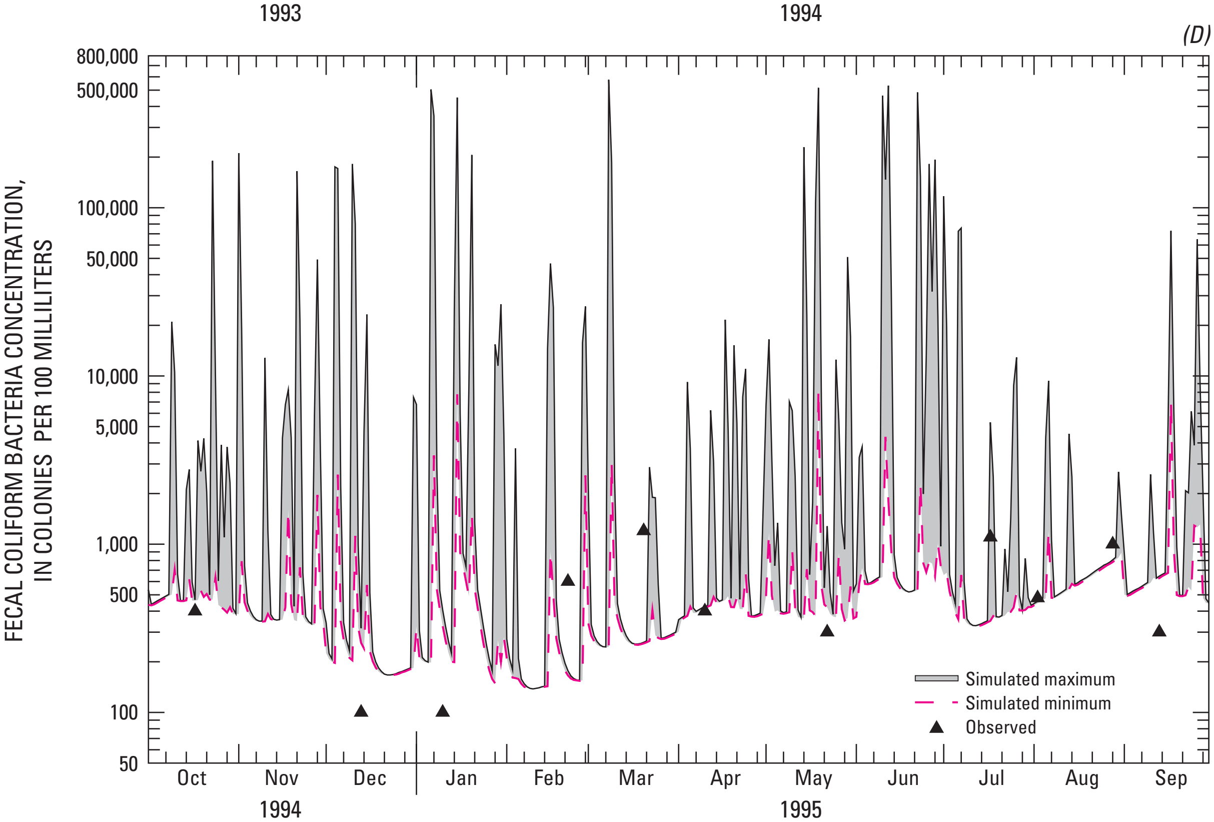


Figure 28. Simulated daily minimum and maximum concentrations, and observed instantaneous concentrations of fecal coliform bacteria at Route 794, water years 1992 *(A)*, 1993 *(B)*, 1994 *(C)*, 1995 *(D)*, 1996 *(E)*, and 1997 *(F)*, Christians Creek, Augusta County, Virginia. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)—Continued

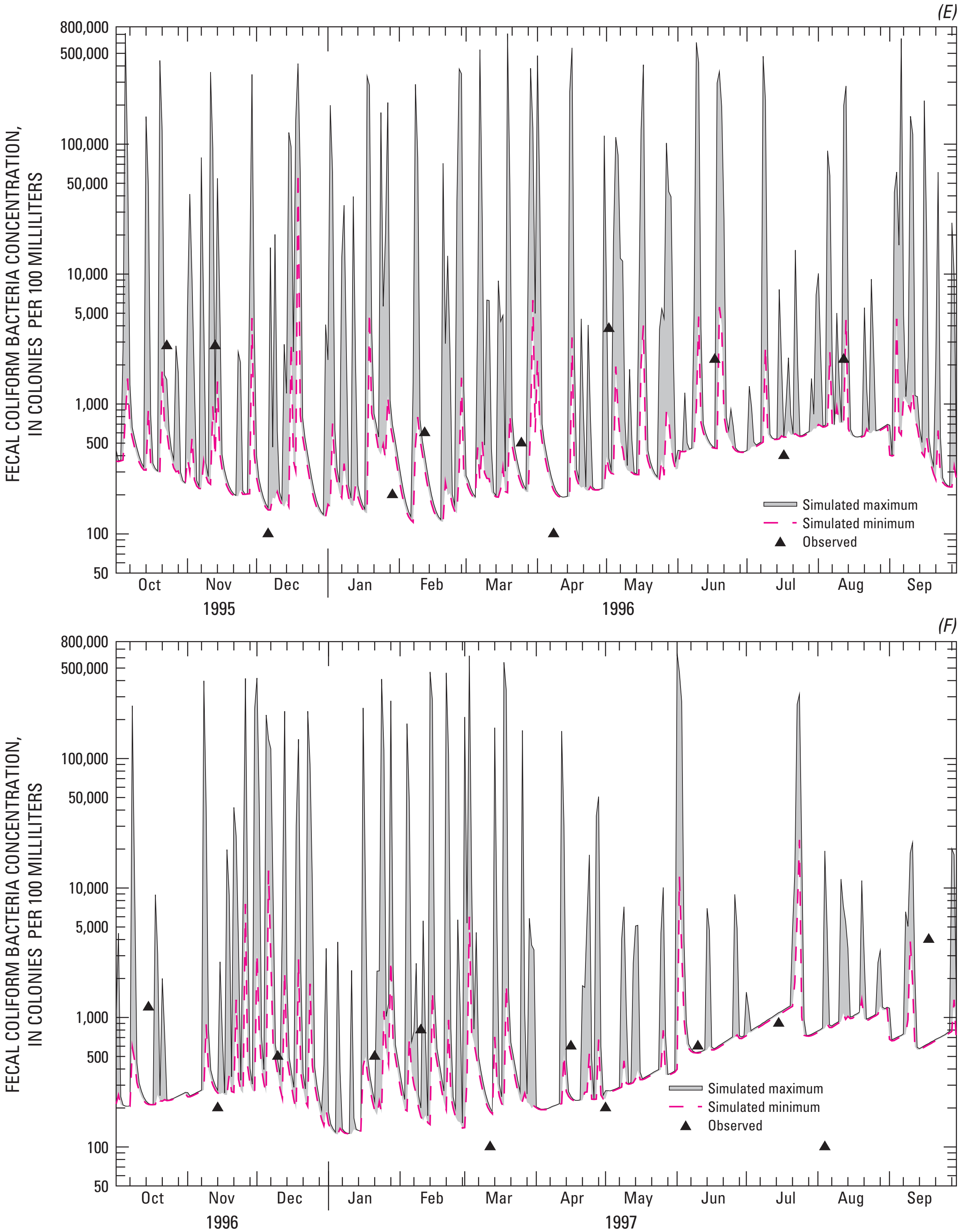


Figure 28. Simulated daily minimum and maximum concentrations, and observed instantaneous concentrations of fecal coliform bacteria at Route 794, water years 1992 *(A)*, 1993 *(B)*, 1994 *(C)*, 1995 *(D)*, 1996 *(E)*, and 1997 *(F)*, Christians Creek, Augusta County, Virginia. (Data from Roderick V. Bodkin, Virginia Department of Environmental Quality, written commun., 1999.)—Continued

coliform concentrations during stormflow periods are similar to simulated concentrations. The simulated recession of fecal coliform concentrations following a storm event ranged from 1 to 7 days (figs. 27 and 28). This range is consistent with the findings from Hyer and Moyer (2003) that elevated fecal coliform concentrations are maintained for 1 to 5 days following a storm event.

The calibrated fecal coliform model also was evaluated by comparing simulated with observed BST data collected at Route 794. These data describe the percent contribution of fecal coliform bacteria from various sources to Christians Creek during an 18-month time period. The mean annual percent contribution to the total instream fecal coliform load from each represented source was simulated using the fecal coliform model. The initial comparison following model calibration between the simulated and observed BST data to observed concentration data revealed that simulated contributions from cattle (68 percent), duck (9 percent), and sheep (9 percent) are overestimated, whereas the simulated contributions from the remaining sources were underestimated (fig. 29A). This initial comparison of simulated and observed BST data revealed that the input sources to the model were not represented accurately. Adjustments were made to the ACCUM values for each source until the simulated BST signature closely approximated the observed BST signature (fig. 29B).

The calibrated fecal coliform model also was evaluated through comparison of the 30-day geometric mean for the simulated fecal coliform bacteria concentrations with the geometric mean of observed (period of record) concentrations. This comparison was a final check on the calibrated fecal coliform model but was not part of the iterative calibration process. The geometric means of the observed fecal coliform data at Route 831 and Route 794 for the period 1991-97 are 1,429 and 558 col/100 mL, respectively. Fecal coliform concentrations generally are higher at the upstream Route 831 site than the Route 794 site. The geometric means of the simulated fecal coliform concentrations at Route 831 and Route 794 are 1,619 and 1,057 col/100 mL, respectively. The pattern in the simulated geometric mean fecal coliform concentrations is similar to the observed concentrations in that the fecal coliform model simulates higher fecal coliform concentrations at the Route 831 site than the Route 794 site.

The simulated geometric mean concentrations at Route 831 and Route 794 are higher than the observed concentrations, primarily because different data sets are used to calculate the simulated and observed geometric means. The simulated geometric mean concentration is calculated using daily mean concentrations of fecal coliform bacteria; thus, elevated concentrations generated during stormflow periods are represented, increasing the geometric mean. The observed geometric mean concentration is calculated using instantaneous monthly concentrations, so that not all of the elevated fecal coliform bacteria concentrations generated during stormflow periods are represented, and the resulting geometric mean is lower. Nonetheless, the comparison between simulated and observed geometric mean concentrations provides additional data on the accuracy of the fecal coliform model for simulating the fate and transport of fecal coliform bacteria in the Christians Creek watershed.

Final Fecal Coliform Model Parameters

WSQOP (rate of surface runoff that results in 90-percent washoff of fecal coliform bacteria in 1 hour) was the only non-source-specific fecal coliform model parameter adjusted during the calibration process. WSQOP was used to adjust the washoff response of the fecal coliform bacteria to rainfall events. Also, WSQOP was used during the calibration of simulated storm peaks. The final calibrated values of WSQOP for each land-use type represented in the model range from 0.2 to 0.6 in. per hour (table 29).

Table 29. Final values of WSQOP used for the land-use types represented in the fecal coliform model for Christians Creek, Augusta County, Virginia

[WSQOP, Rate of surface runoff required to remove 90 percent of the surface-stored fecal coliform bacteria]

Land-use types	WSQOP (inch per hour)
Urban	0.3
Residential	.3
Cropland	.5
Hayland	.5
Pasture	.5
Forest	.6
Urban impervious	.2

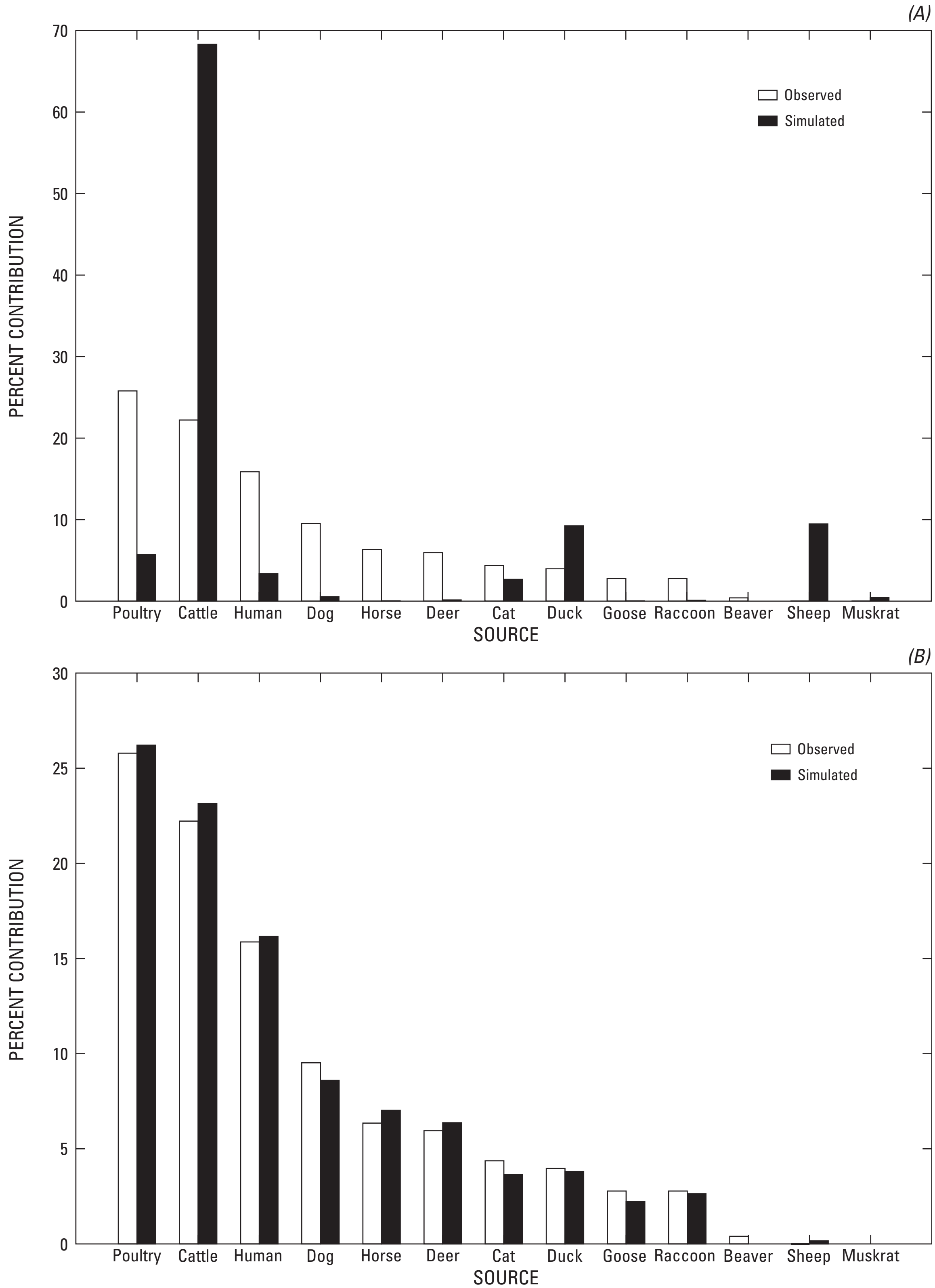


Figure 29. Observed and simulated percent contribution from the simulated sources in the watershed to the total instream fecal coliform bacteria load at Route 794, initial calibration *(A)*, and final calibration *(B)*, Christians Creek, Augusta County, Virginia.

The two source-specific model parameters adjusted during the calibration process were the fecal coliform accumulation rate on the land surface (ACCUM) and the limit of storage of fecal coliform bacteria on the land surface (SQOLIM). ACCUM for each source was manipulated during calibration; SQOLIM was maintained at 9 times ACCUM. The total fecal coliform contributions from humans, dogs, and cats were calibrated by adjusting their initial estimated population (POPN) values (table 30). The percentage of dogs depositing their feces on impervious areas was decreased from 10 percent to 1 percent. ACCUM values for cattle, poultry, horses, and sheep were calibrated by adjusting FCden (number of bacteria per gram of feces produced) (table 31). ACCUM for deer was calibrated by adjusting the FCden, whereas ACCUM values for geese, ducks, raccoons, muskrats, and beavers were calibrated through adjustments to POPN (table 32). POPN values for humans, dogs, cats, geese, ducks, raccoons, muskrats, and beavers are a result of model calibration and represent the populations to account for the uncertainty associated with the fixed values of Fprod, FCden, and habitat area (HAB); POPN values do not represent the actual populations in the watershed.

FECAL COLIFORM TMDL

Present Conditions

The simulated fecal coliform bacteria concentrations in Christians Creek, water years 1991-97, were converted to 30-day geometric mean concentrations. The 30-day geometric mean concentrations indicate that predicted fecal coliform concentrations at both Route 831 and Route 794 exceed the State geometric mean water-quality standard of 200 col/100 mL (fig. 30A). Based on the peak fecal coliform 30-day geometric mean concentrations of 3,448 and 6,160 col/100 mL at Route 794 and Route 831, respectively, an approximately from 94- to 97-percent reduction of the current instream fecal coliform load is needed to meet the designated water-quality standard.

Most of the fecal coliform load (99.6 percent) entering Christians Creek is from nonpoint sources in the watershed (table 33). Thus, most of the fecal coliform bacteria are transported during stormflow periods. However, the incorporation of a geometric mean calculation and the need for compliance with the geometric mean water-quality standard places a greater emphasis on base-flow conditions that are dominated by point source contributions. The geometric mean calculation is used to identify an unbiased average in the presence of outliers, such as elevated concentrations of fecal coliform bacteria associated with stormflow events. In order to meet the State water-quality standard, reductions are needed in fecal coliform loads from both nonpoint sources and sources depositing directly in the streams.

Scenarios for Fecal Coliform Load Reductions

Total instream fecal coliform load reductions of approximately 94–97 percent will reduce the observed fecal coliform concentrations below the State water-quality standard and designated 5-percent MOS (30-day geometric mean of 190 col/100 mL). Three source-load reduction scenarios for meeting the water-quality goals for Christians Creek were developed through discussions including DCR, DEQ, USGS (in a technical advisory role) and local stakeholders (table 34). These scenarios feature source-specific reductions in fecal coliform loads from nonpoint sources and point sources, including direct deposition from cattle in streams and straight pipes. Scenario 1 requires a 100-percent reduction in the present fecal coliform loading from cattle, poultry, sheep, horses, humans, dogs and cats (nonpoint sources), a 90-percent reduction in fecal coliform loading from parking lots and roads, and a 100-percent reduction in the fecal coliform loading from cattle in streams and straight pipes (point sources) in order to ensure that the State water-quality standard is not exceeded. Scenarios 2 and 3 require greater reductions in fecal coliform loading from wildlife sources (50 and 94 percent, respectively) and parking lots and roads (100 and 99 percent, respectively), whereas lesser reductions are needed from the livestock and pet sources in order to ensure that the State water-quality standard is not exceeded. These three scenarios were discussed and evaluated in a public review process led by DCR and DEQ, and scenario 3 was chosen for the Christians Creek watershed.

After the source-load reduction strategies in scenario 3 were incorporated into the watershed model, simulated fecal coliform concentrations at both Route 831 and Route 794 met the water-quality goals (fig. 30B). Simulated fecal coliform concentrations at the mouth of Christians Creek (reach 6, fig. 31) also

Table 30. Final values of the total amount of feces produced daily and fecal coliform per gram of feces generated by the human, dog and cat populations in the residential hydrologic response unit represented in the fecal coliform model, Christians Creek, Augusta County, Virginia

[Fprod, feces produced per day; FCden, fecal coliform bacteria per gram of feces; POPN, population size; HAB, habitat area; –, not applicable]

Subwatershed[1]	Fprod (grams)	FCden	POPN (number)		HAB (acres)	
			Residential	Urban	Residential	Urban
			Human–Land Applied			
1	150	4.66×10^8	272	–	289	–
2	150	4.66×10^8	176	–	206	–
3	150	4.66×10^8	339	–	309	–
4	150	4.66×10^8	224	–	485	–
5	150	4.66×10^8	330	–	808	–
6	150	4.66×10^8	209	–	261	–
			Human–Straight Pipes			
1	150	4.66×10^8	3	–	–	–
2	150	4.66×10^8	3	–	–	–
3	na	–	–	–	–	–
4	150	4.66×10^8	3	–	–	–
5	na	–	–	–	–	–
6	150	4.66×10^8	3	–	–	–
			Dog			
1	450	4.11×10^6	3,121	1,860	289	414
2	450	4.11×10^6	2,019	1,202	206	153
3	450	4.11×10^6	3,887	2,316	309	495
4	450	4.11×10^6	2,567	1,530	485	1,244
5	450	4.11×10^6	3,784	2,255	808	196
6	450	4.11×10^6	2,398	1,429	261	429
			Dog-Impervious			
1	450	4.11×10^6	–	19	–	26
2	450	4.11×10^6	–	12	–	10
3	450	4.11×10^6	–	23	–	32
4	450	4.11×10^6	–	15	–	170
5	450	4.11×10^6	–	23	–	13
6	450	4.11×10^6	–	14	–	27
			Cat			
1	20	1.49×10^7	8,291	4,990	289	414
2	20	1.49×10^7	5,362	3,228	206	153
3	20	1.49×10^7	10,324	6,215	309	495
4	20	1.49×10^7	6,818	4,105	485	1,244
5	20	1.49×10^7	10,051	6,051	808	196
6	20	1.49×10^7	6,370	3,835	261	429

[1]See figure 3 for location of subwatersheds.

Table 31. Final values of the total amount of feces produced daily and fecal coliform bacteria per gram of feces generated by cattle, poultry, horses, and sheep represented in the fecal coliform model, Christians Creek, Augusta County, Virginia

[Fprod, feces produced per day; FCden, fecal coliform bacteria per gram of feces]

Source	Average daily Fprod (grams)	FCden
Dairy cattle	54,545	1.98×10^5
Beef cattle	20,909	5.60×10^5
Heifers	39,091	1.41×10^6
Poultry	231	1.83×10^9
Horse	23,182	4.44×10^6
Sheep	1,091	1.80×10^6

Table 32. Final values for population, total amount of feces produced daily and fecal coliform bacteria per gram of feces for deer, goose, duck, raccoon, muskrat, and beaver represented in the watershed model, Christians Creek, Augusta County, Virginia

[Fprod, feces produced per day; FCden, fecal coliform bacteria per gram of feces; POPN, population size; F, Forest; P, Pasture; U, Urban; R, Residential; H, Hayland; C, Cropland]

Wildlife source	Land-use type	Population density (number per acre)	POPN (number)	Fprod (grams)	FCden
Deer	F, P	0.040	975	772	2.24×10^8
Goose–Summer	U, R, P, H, C	4.87	23,293	225	3.55×10^6
Goose–Winter	U, R, P, H, C	6.23	30,359	225	3.55×10^6
Duck–Summer	U, R, P, H, C	.71	3,395	150	4.90×10^7
Duck–Summer	F	.19	413	150	4.90×10^7
Duck–Winter	U, R, P, H, C	1.50	7,175	150	4.90×10^7
Duck–Winter	F	.31	674	150	4.90×10^7
Raccoon	F	.22	4308	450	1.11×10^7
Raccoon	R, P, H, C	.09	3821	450	1.11×10^7
Muskrat	U, R, P, H, C, F	.500	479	100	2.50×10^5
Beaver	F	.016	5	200	1.00×10^3
Beaver	U, R, P, H, C	.008	5	200	1.00×10^3

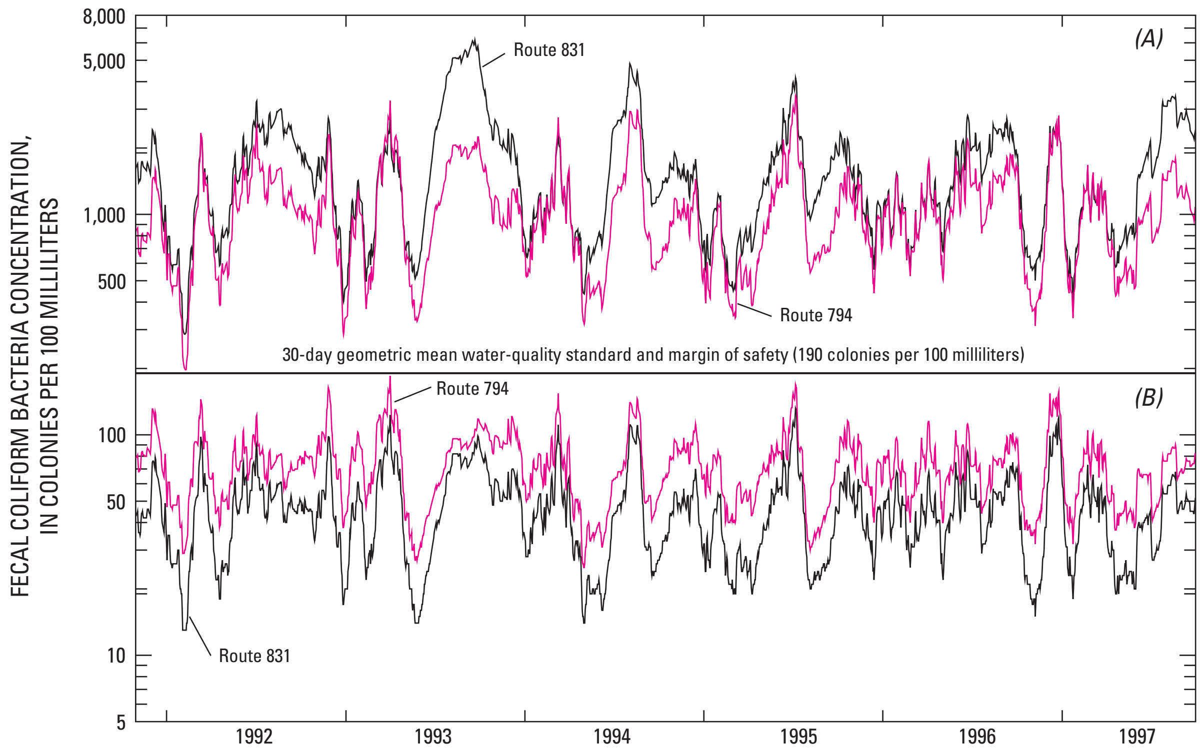


Figure 30. Simulated 30-day geometric mean fecal coliform concentrations before *(A)* and after *(B)* incorporation of the Total Maximum Daily Load (TMDL) allocation scenario at Route 831 and Route 794 for Christians Creek, Augusta County, Virginia, water years 1992-97.

will meet the water-quality goals following implementation of scenario 3, thus bringing Christians Creek into compliance with the State water-quality standard from the headwaters to the mouth. Changes to the present fecal coliform load allocation following the incorporation of the source-specific load reductions in scenario 3 are in table 35. Average annual fecal coliform loading pre- and post-TMDL allocations are 3.69 x 10^{16} and 1.39 x 10^{15} col/year, respectively. The percent reductions in the fecal coliform load delivered from the various land types ranged from 94 to 99 percent as a result of the reduction scenario. The needed percent reduction in the fecal coliform load delivered from cattle in

Table 33. Total annual load of fecal coliform bacteria delivered from the various land-use types, direct deposition by cattle and humans and permitted discharges for present conditions in Christians Creek, Augusta County, Virginia

Land-use types	Total annual load of fecal coliform bacteria for present conditions (colonies per year)	Contribution (percent)
Urban	2.54 x 10^{15}	6.88
Residential	1.01 x 10^{16}	27.34
Pasture	1.15 x 10^{16}	31.14
Cropland	7.05 x 10^{15}	19.09
Hayland	4.17 x 10^{15}	11.29
Forest	1.38 x 10^{15}	3.74
Urban Impervious	4.92 x 10^{13}	.13
Point Sources		
Instream deposition from cattle and humans	1.44 x 10^{14}	.39
Permitted discharges	2.36 x 10^{12}	.01
Total	3.69 x 10^{16}	100

Table 34. Scenarios for reducing fecal coliform bacteria loads and associated percent reductions from nonpoint and point sources represented in the fecal coliform model for Christians Creek, Augusta County, Virginia

Scenario number	Percent reduction from present fecal coliform load																	Average 30-day geometric mean concentration of fecal coliform bacteria (colonies per 100 milliliters)
	Nonpoint sources														Point sources			
	Cattle	Poultry	Sheep	Horse	Human	Dog	Cat	Goose	Duck	Deer	Raccoon	Muskrat	Beaver	Parking lots and roads	Cattle in streams	Straight pipes	Permitted discharges	
1	100	100	100	100	100	100	100	0	0	0	0	0	0	90	100	100	0	9
2	100	100	100	100	100	100	100	50	50	50	0	0	0	100	98	100	0	41
3	94	94	94	94	100	94	94	94	94	94	94	0	0	99	99	100	0	73

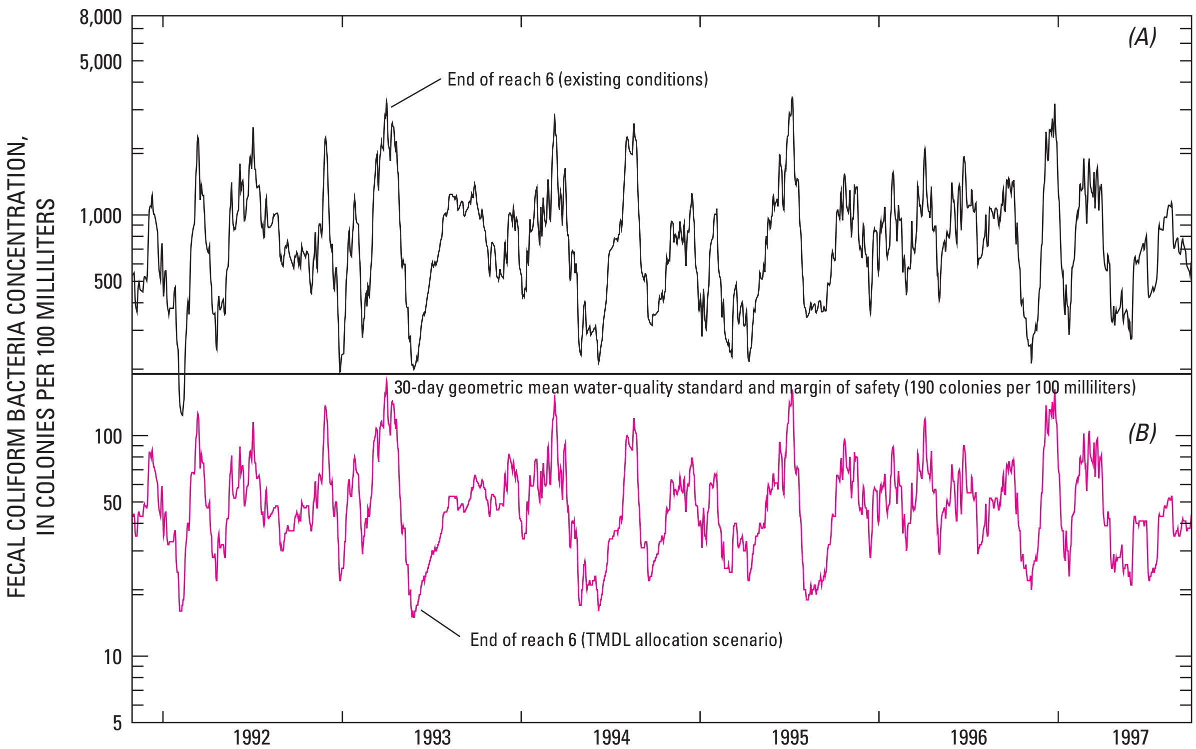


Figure 31. Simulated 30-day geometric mean fecal coliform concentrations before *(A)* and after *(B)* incorporation of the Total Maximum Daily Load (TMDL) allocation scenario at Route 831 and Route 794 at the end of storm reach 6 for Christians Creek, Augusta County, Virginia, water years 1992-97.

Table 35. Total annual loads of fecal coliform bacteria delivered from the land-use types, point sources, and permitted discharges for present conditions and after incorporation of total maximum daily load (TMDL) allocation in Christians Creek, Augusta County, Virginia

[NC, no change]

Land-use types	Total annual load of fecal coliform bacteria for present conditions (colonies per year)	Total annual load after incorporation of TMDL (colonies per year)	Reduction (percent)
Urban	2.54×10^{15}	1.01×10^{14}	96.01
Residential	1.01×10^{16}	1.23×10^{14}	98.78
Pasture	1.15×10^{16}	4.96×10^{14}	95.69
Cropland	7.05×10^{15}	4.28×10^{14}	93.94
Hayland	4.17×10^{15}	1.66×10^{14}	96.01
Forest	1.38×10^{15}	5.81×10^{13}	95.79
Urban impervious	4.92×10^{13}	3.21×10^{11}	99.35
Point Sources			
Instream deposition from cattle and humans	1.44×10^{14}	1.12×10^{12}	99.22
Permitted discharges	2.36×10^{12}	1.18×10^{13}	NC
Total	3.69×10^{16}	1.39×10^{15}	96.23

streams and straight pipes equaled 99 percent while no reduction in fecal coliform loading from permitted dischargers (sewage treatment plants) was required. The resulting TMDL equation (see eq. 1) that meets the fecal coliform bacteria water-quality goals for Christians Creek is

$$1.46 \times 10^{15} \text{ col/yr } (TMDL) = 1.18 \times 10^{13} \text{ col/yr } (\sum WLAs) + 1.38 \times 10^{15} \text{ col/yr } (\sum LAs) + 6.96 \times 10^{13} \text{ col/yr } (MOS).$$

Attaining the designated water-quality goals for Christians Creek is a three-step process:

(1) Determination of the fecal coliform bacteria TMDL for Christians Creek.

(2) Development of a plan for reducing the current fecal coliform loading to Christians Creek.

(3) Implementation of the source-load reduction strategies and follow-up monitoring to ensure that the TMDL plan and implementation result in achievement of the water-quality goals for Christians Creek.

DIRECTIONS FOR FUTURE RESEARCH

This study demonstrated the utility of incorporating both HSPF and BST data into the process of developing a TMDL for fecal coliform bacteria. This process would be enhanced by continued refinement of BST techniques and research in the following areas:

- The range of fecal coliform densities for various warm-blooded species and how this range varies temporally and spatially.
- The effect of sediment on the transport and storage of fecal coliform bacteria.
- The fate and transport of fecal coliform bacteria in the shallow subsurface (both the unsaturated zone and the shallow aquifer system) and potential contributions to the instream fecal coliform load.

SUMMARY

The U.S. Geological Survey (USGS), in cooperation with the Virginia Department of Conservation and Recreation (DCR), began a 3-year study in 1999 to develop a total maximum daily load (TMDL) for fecal coliform bacteria in the Christians Creek watershed. The Virginia Department of Environmental Quality (DEQ) determined that Christians Creek is impaired by fecal coliform bacteria because of violations of the State water-quality standard (1,000 colonies/100 mL). This study demonstrates the utility of incorporating both watershed modeling using Hydrological Simulation Program–FORTRAN (HSPF) and bacterial source tracking (BST) as tools in the development of a fecal coliform bacteria TMDL. Attaining the designated water-quality goals for Christians Creek involves a three-step process, determined by DCR and DEQ, which is (1) determination of the fecal coliform TMDL, (2) development of a plan for reducing the current fecal coliform loading, and (3) implementation of the source-load reduction strategies and follow-up water-quality monitoring. Specific objectives of this study were to (1) produce calibrated models of watershed streamflow and fecal coliform bacteria transport, (2) incorporate BST information into the fecal coliform model calibration process, (3) estimate fecal coliform source-load reductions required to meet the State water-quality standard, and (4) define the TMDL for fecal coliform bacteria for Christians Creek. The major findings and conclusions of the study are:

- The calibrated streamflow model simulated observed streamflow characteristics with respect to total annual runoff, seasonal runoff, average daily streamflow, and hourly stormflow.
- BST identified that the major contributors of fecal coliform bacteria in Christians Creek are poultry, cattle, humans, dogs, horses, and deer.
- The calibrated fecal coliform model simulated the patterns and range of fecal coliform bacteria concentrations observed by DEQ (1991-97) and USGS (1999-2000).
- The calibrated fecal coliform model simulated source-specific instream fecal coliform loads comparable to the source-specific percent contribution identified in Christians Creek by BST.
- Incorporation of BST data reduces the uncertainty associated with determining source-specific fecal coliform loading in the watershed.
- A 96-percent reduction in the current fecal coliform load delivered to Christians Creek is required to meet the designated water-quality goals and associated TMDL.

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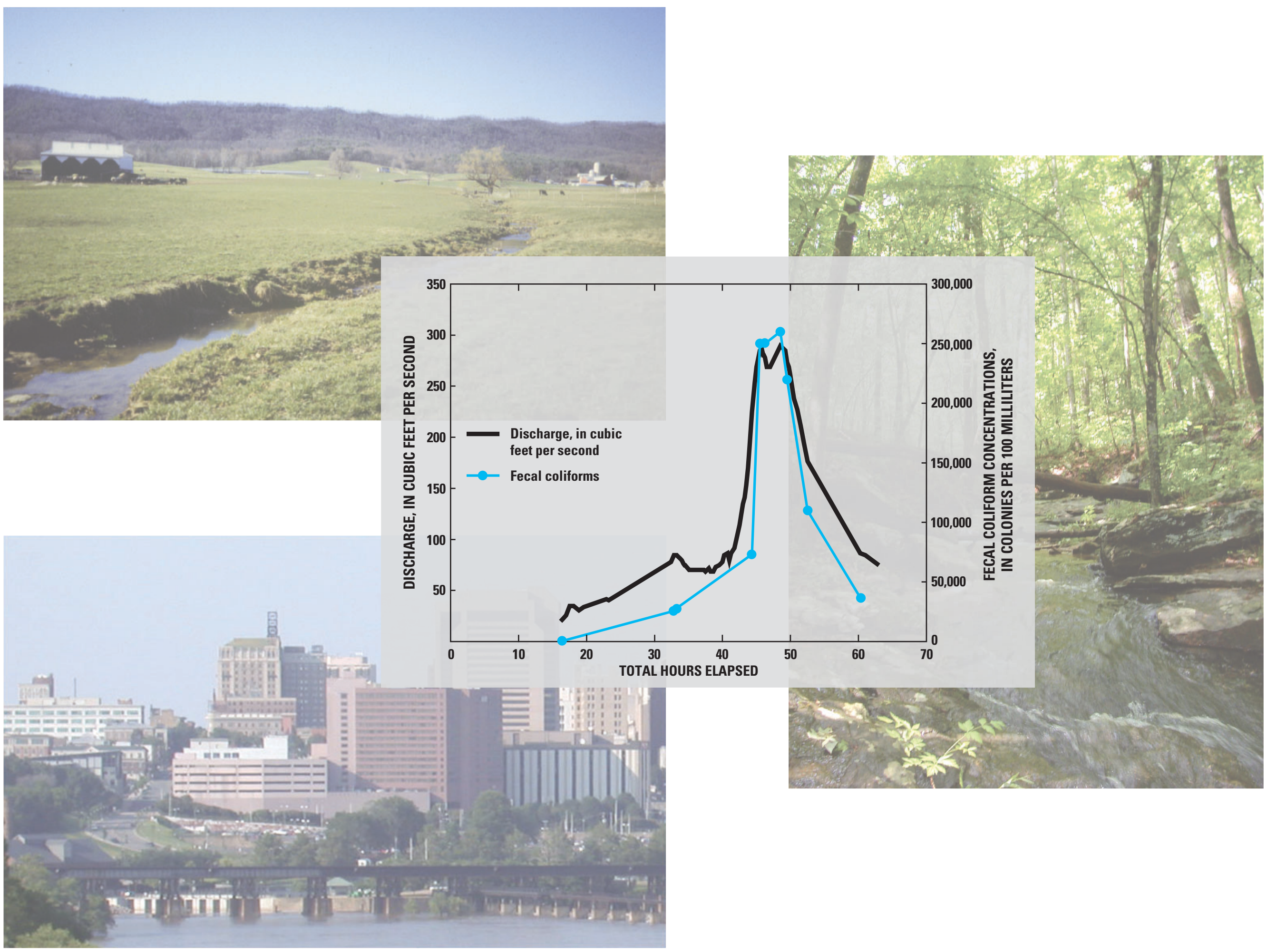

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Representative agricultural, urban, and forested streams (Photographs by K.E. Hyer and D.L. Moyer, U.S. Geological Survey)

U.S. Department of the Interior
U.S. Geological Survey

Patterns and Sources of Fecal Coliform Bacteria in Three Streams in Virginia, 1999-2000

By Kenneth E. Hyer and Douglas L. Moyer

Water-Resources Investigations Report 03-4115

Prepared in cooperation with:

Virginia Department of Conservation and Recreation
Virginia Department of Environmental Quality
Fairfax County, Virginia

Richmond, Virginia
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CONVERSION FACTORS, DATUM, AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
Length		
inch (in.)	25.4	millimeter
foot (ft)	0.3048	meter
mile (mi)	1.609	kilometer
Area		
square mile (mi^2)	259.0	hectare
square mile (mi^2)	2.590	square kilometer
Volume		
gallon (gal)	3.785	liter
gallon (gal)	0.003785	cubic meter
Flow		
cubic foot per second (ft^3/s)	0.02832	cubic meter per second
gallons per day	0.003785	cubic meter per day
million gallons per day (Mgal/d)	0.04381	cubic meter per second

Horizontal coordinate information is referenced to the North American Datum of 1927 (NAD27).

Temperature: Temperature is reported in degrees Celsius (°C), which can be converted to degrees Fahrenheit (°F) as follows: °F = 1.8 (°C) + 32°

Abbreviated water-quality units: Chemical concentration is reported in milligrams per liter (mg/L) or micrograms per liter (μg/L). Milligrams per liter is a unit expressing the concentration of chemical constituents in solution as mass (milligrams) of solute per unit volume (liter) of water. One-thousand micrograms per liter is equivalent to 1 milligram per liter. For concentrations less than 7,000 mg/L, the numerical value is the same as for concentrations in parts per million. Bacterial concentrations are reported in units of colonies per 100 milliliters (col/100mL). Specific electrical conductance of water is reported in microsiemens per centimeter at 25 degrees Celsius (μS/cm). Turbidity is reported in nephelometric turbidity units (NTU).

Patterns and Sources of Fecal Coliform Bacteria in Three Streams in Virginia, 1999-2000

By Kenneth E. Hyer and Douglas L. Moyer

ABSTRACT

Surface-water impairment by fecal coliform bacteria is a water-quality issue of national scope and importance. In Virginia, more than 175 stream segments are on the Commonwealth's 1998 303(d) list of impaired waters because of elevated concentrations of fecal coliform bacteria. These fecal coliform-impaired stream segments require the development of total maximum daily load (TMDL) and associated implementation plans, but accurate information on the sources contributing these bacteria usually is lacking. The development of defendable fecal coliform TMDLs and management plans can benefit from reliable information on the bacteria sources that are responsible for the impairment. Bacterial source tracking (BST) recently has emerged as a powerful tool for identifying the sources of fecal coliform bacteria that impair surface waters. In a demonstration of BST technology, three watersheds on Virginia's 1998 303(d) list with diverse land-use practices (and potentially diverse bacteria sources) were studied. Accotink Creek is dominated by urban land uses, Christians Creek by agricultural land uses, and Blacks Run is affected by both urban and agricultural land uses. During the 20-month field study (March 1999–October 2000), water samples were collected from each stream during a range of flow conditions and seasons. For each sample, specific conductance, dissolved oxygen concentration, pH, turbidity, flow, and water temperature were measured. Fecal coliform concentrations of each water sample were determined using the membrane filtration technique. Next, *Escherichia coli* (*E. coli*) were isolated from the fecal coliform bacteria and their sources were identified using ribotyping (a method of "genetic fingerprinting").

Study results provide enhanced understanding of the concentrations and sources of fecal coliform bacteria in these three watersheds. Continuum sampling (sampling along the length of the streams) indicated that elevated concentrations of fecal coliform bacteria (maximum observed concentration of 290,000 colonies/100 milliliters (col/100mL) could occur along the entire length of each stream, and that the samples collected at the downstream monitoring station of each stream were generally representative of the entire upstream reach. Seasonal patterns were observed in the base-flow fecal coliform concentrations of all streams; concentrations were typically highest in the summer and lowest in the winter. Fecal coliform concentrations were lowest during periods of base flow (typically 200–2,000 col/100mL) and increased by 3–4 orders of magnitude during storm events (as high as 700,000 col/100mL). Multiple linear regression models were developed to predict fecal coliform concentrations as a function of streamflow and other water-quality parameters. The source tracking technique provided identification of bacteria contributions from diverse sources that included (but were not limited to) humans, cattle, poultry, horses, dogs, cats, geese, ducks, raccoons, and deer. Seasonal patterns were observed in the contributions of cattle and poultry sources. There were relations between the identified sources of fecal coliform bacteria and the land-use practices within each watershed. There were only minor differences in the distribution of bacteria sources between low-flow periods and high-flow periods. A coupled approach that utilized both a large available source library and a smaller, location-specific source library provided the most success in identifying the unknown *E. coli* isolates. BST data should provide valuable support and guidance for producing more defend-

able and scientifically rigorous watershed models. Incorporation of these bacteria-source data into watershed management strategies also should result in the selection of more efficient source-reduction scenarios for improving water quality.

INTRODUCTION

Surface-water impairment by fecal coliform bacteria is a water-quality issue of national scope and importance. Section 303(d) of the Clean Water Act requires that each State identify surface waters that do not meet applicable water-quality standards. In Virginia, more than 175 stream segments are on the Commonwealth's 1998 303(d) list of impaired waters because of violations of the fecal coliform bacteria standard (an instantaneous water-quality standard of 1,000 col/100 mL, or a geometric mean water-quality standard of 200 col/100 mL). Fecal coliform concentrations that violate either standard indicate an increased risk to human health when these waters are contacted through swimming or other recreational activities.

In Virginia, total maximum daily load (TMDL) plans will need to be developed over the next 10 years for all impaired water bodies identified on the State's 1998 303(d) list. TMDL plans provide a quantitative representation of all the contaminant contributions to a stream:

$$TMDL = \sum WLAi + \sum LAi + MOS \tag{1}$$

where $\sum WLAi$ represents the sum of all the point-source loadings, $\sum LAi$ represents the sum of all the nonpoint-source loadings, and MOS represents a margin of safety. The sum of these loading terms and assigned margin of safety constitutes the TMDL and represents the fecal coliform loading that the surface-water body can assimilate without violating the state's water-quality standards. For a TMDL plan to be approved by the U.S. Environmental Protection Agency (USEPA), all major fecal coliform contributions to the stream must be identified and quantified. Once a TMDL plan is established, fecal coliform source-load contributions are then reduced (through implementation of source-control management practices) until the target TMDL is achieved.

Establishing TMDLs in waters contaminated by fecal coliform bacteria is difficult because the specific sources of the bacteria are numerous and the magnitude of their contributions is commonly unknown. Potential sources of fecal coliform bacteria include all warm-blooded animals (humans, pets, domesticated livestock, birds, and wildlife). The lack of information on bacteria sources makes it difficult to develop accurate load allocations, technically defensible TMDLs, and appropriate source-load reduction measures. Information about the major fecal coliform sources that impair surface-water quality would represent a major improvement in the development of technically defensible TMDLs.

Bacterial source tracking (BST) recently has emerged as a tool for identifying the sources of fecal coliform bacteria that impair surface waters. In application, this technology identifies specific differences among the fecal coliform bacteria that are present in the feces of different animal species. Time, diet, environment, and many other factors may have contributed to produce these evolutionary distinctions; these distinctions are used in BST to identify the animal source of fecal coliform bacteria that have been isolated from a waterbody.

BST is a rapidly growing technology with various analytical techniques available, depending on the goals of the study. In general, these techniques rely on molecular, genetics-based approaches (also known as "genetic fingerprinting"), or phenotypic (relating to the physical characteristics of an organism) distinctions between the bacteria of different sources. Three primary genetic techniques are available for BST. Ribotyping characterizes a small, specific portion of the bacteria's DNA sequence (Samadpour and Chechowitz, 1995). Pulsed-field gel electrophoresis (PFGE) is similar to ribotyping but typically is performed on the entire genome of the bacteria (Simmons and others, 1995). Polymerase chain reaction (PCR) amplifies selected DNA sequences in the bacteria's genome (Makino and others, 1999). Phenotypic techniques generally involve an antibiotic resistance analysis, where resistance patterns for a suite of different concentrations and types of antibiotics are developed (Wiggins, 1996; Hagedorn and others, 1999).

Although all these techniques show promise for bacteria source identification, the ribotyping technique was chosen for this study. Ribotyping involves an analysis of the specific DNA sequence that codes for the production of ribosomal RNA (ribonucleic acid). Ribotyping has been demonstrated to be an effective technique for distinguishing bacteria from the feces of multiple animal sources (Carson and others, 2001); it has been performed successfully and used to identify fecal coliform bacteria sources in both freshwater (Samadpour and Chechowitz, 1995) and estuarine systems (Ongerth and Samadpour, 1994). Furthermore, the technique has been used to identify the sources of bacteria contributing to impairments in both urban (Herrera Environmental Consultants, Inc., 1993) and wilderness systems (Farag and others, 2001). The broad applicability of ribotyping makes it well suited for use in this study.

This study was performed to demonstrate the field application of BST technology and to identify the sources of fecal coliform bacteria in three streams on Virginia's 1998 303(d) list of impaired waters. The three streams sampled during this study were selected because they represent a range of land uses (urban, agricultural, and mixed urban/agricultural) and most of the potential fecal coliform sources that are likely to be encountered throughout the Commonwealth. The three streams were sampled over a period of 20 months (March 1999–October 2000) and over a wide range of hydrological conditions. For all samples, the fecal coliform concentration, specific conductance, turbidity, pH, water temperature, and dissolved oxygen concentration were determined. Ribotyping was used to identify the sources of the fecal coliform bacteria. The results of this study have broad implications for the development of fecal coliform watershed models, selection of TMDL allocation scenarios, and the identification of effective strategies for reducing fecal coliform contributions to streams. The U. S. Geological Survey (USGS) conducted this study in cooperation with the Virginia Department of Environmental Quality (DEQ), Virginia Department of Conservation and Recreation (DCR), and Fairfax County, Virginia.

Purpose and Scope

This report demonstrates the field application of bacterial source tracking technology, which was used to identify the sources of fecal coliform bacteria in three streams that are on Virginia's 1998 303(d) list of impaired waters. Streamwater data were collected from March 1999 through October 2000, under both base-flow and storm-flow conditions. Concentrations of fecal coliform bacteria were determined at the stream gage and 4–5 other locations in each watershed; bacterial source tracking was performed only on the samples that were collected at the stream gage in each watershed. In addition to identifying the sources of fecal coliform bacteria in the three streams, the report describes (1) seasonal and discharge-related patterns in the concentrations of fecal coliform bacteria, (2) multiple linear regression models for predicting fecal coliform concentrations as a function of supporting water-quality field parameters, (3) seasonal and discharge-related patterns in the identified bacteria sources of each stream, and (4) the effect of source-library size on the identification of bacteria. Study results have broad implications for the interpretation of source-tracking data and the development of TMDL plans in impaired streams.

Acknowledgments

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DESCRIPTION OF THE STUDY AREAS

Three stream segments on Virginia's 1998 303(d) list were selected for this study. The streams in Virginia that are impaired by fecal coliform bacteria drain watersheds that generally can be categorized into one of three land-use practices: agricultural, urban, and mixed urban/agricultural. To represent a range of land uses and potential sources of fecal contamination, a representative study site was selected from each of these land-use types. The criteria evaluated for site selection included (1) presence of a stream gage, (2) size of watershed (about 100 mi^2 or smaller), (3) well-defined and stable land-use patterns, (4) availability of historical water-quality data, (5) availability of up-to-date geographic information system (GIS) coverages, and (6) support from the local community. The three sites selected for this study (fig. 1) were Accotink Creek (representing urban land use), Christians Creek (agricultural land use), and Blacks Run (mixed urban and agricultural land use). The data collected during this study are being used in a separate watershed modeling and TMDL development study by the USGS (Moyer and Hyer, in press).

Accotink Creek

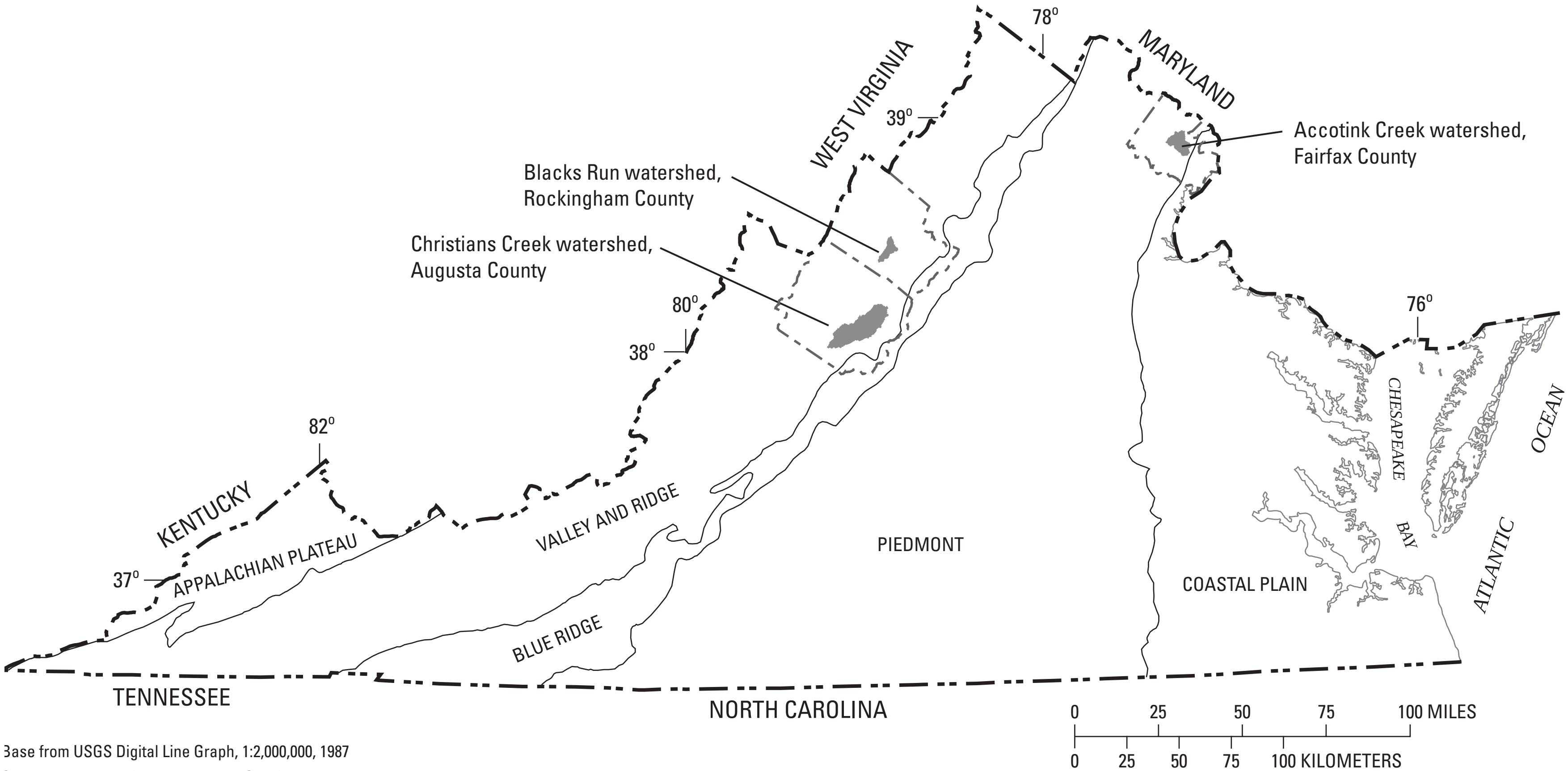
Accotink Creek near Annandale, Va., is the urban watershed selected for this study (fig. 2). The headwaters of Accotink Creek are in the city of Fairfax, Va., and the creek flows for approximately 10.9 mi before it drains into Lake Accotink, located in Fairfax County. The impaired stream reach is a 4.5-mi-long section just upstream of Lake Accotink. The portion of the Accotink Creek watershed studied has a drainage basin area of 25 mi^2 and a population of more than 110,000 (2000 U.S. Census Bureau data). Approximately 600 ft upstream from the bridge at Route 620 (Braddock Road) is a stream gage that has been active since 1949 and is managed by DEQ (USGS station number 01654000). DEQ has performed quarterly sampling for fecal coliform bacteria at the bridge at Route 620 since 1990. Currently, there are no permitted fecal coliform point source dischargers within the watershed (J. Crowther, Virginia Department of Environmental Quality, written commun., 1999).

Although portions of the watershed are forested (especially adjacent to the stream), urban and residential land uses dominate the majority of the watershed. Potential sources of fecal contamination in this urban watershed include domestic pets (such as dogs and cats), wildlife (such as raccoons, opossum, rats, squirrels, and deer), waterfowl (such as geese, ducks, and sea gulls), and humans (as contributed by cross-pipes, leaking or overflowing sewer lines, and failing septic systems).

The Accotink Creek watershed lies in the Piedmont physiographic province, and is underlain by crystalline igneous and metamorphic rocks (Froelich and Zenone, 1985). The surficial geology of the watershed is composed of five formations. The Wissahickon Formation dominates the watershed and is composed of quartz-mica schist, phyllite, and quartzite (Johnston, 1964). The Greenstone Contact Complex is present in some headwater areas of the catchment and is composed of chlorite schist, sericite-chlorite schist, chlorite-quartz schist, talc schist and small amounts of quartzite (Johnston, 1962). Granitic rocks are distributed throughout the watershed; these rocks are of variable composition and include biotite granite, muscovite granite, biotite-muscovite granite, granodiorite, quartz monzonite, and quartz diorite (Johnston, 1964). A small portion of the watershed is underlain by the Sykesville Formation, which includes muscovite or sericite-biotite-quartz schist and gneiss, quartzite, epidote quartzite, and muscovite-biotite quartzite (Johnston, 1964). Alluvial material (composed of clay and sand, as well as quartz cobbles and pebbles) also is present along the channel and in the floodplain of Accotink Creek (Johnston, 1962).

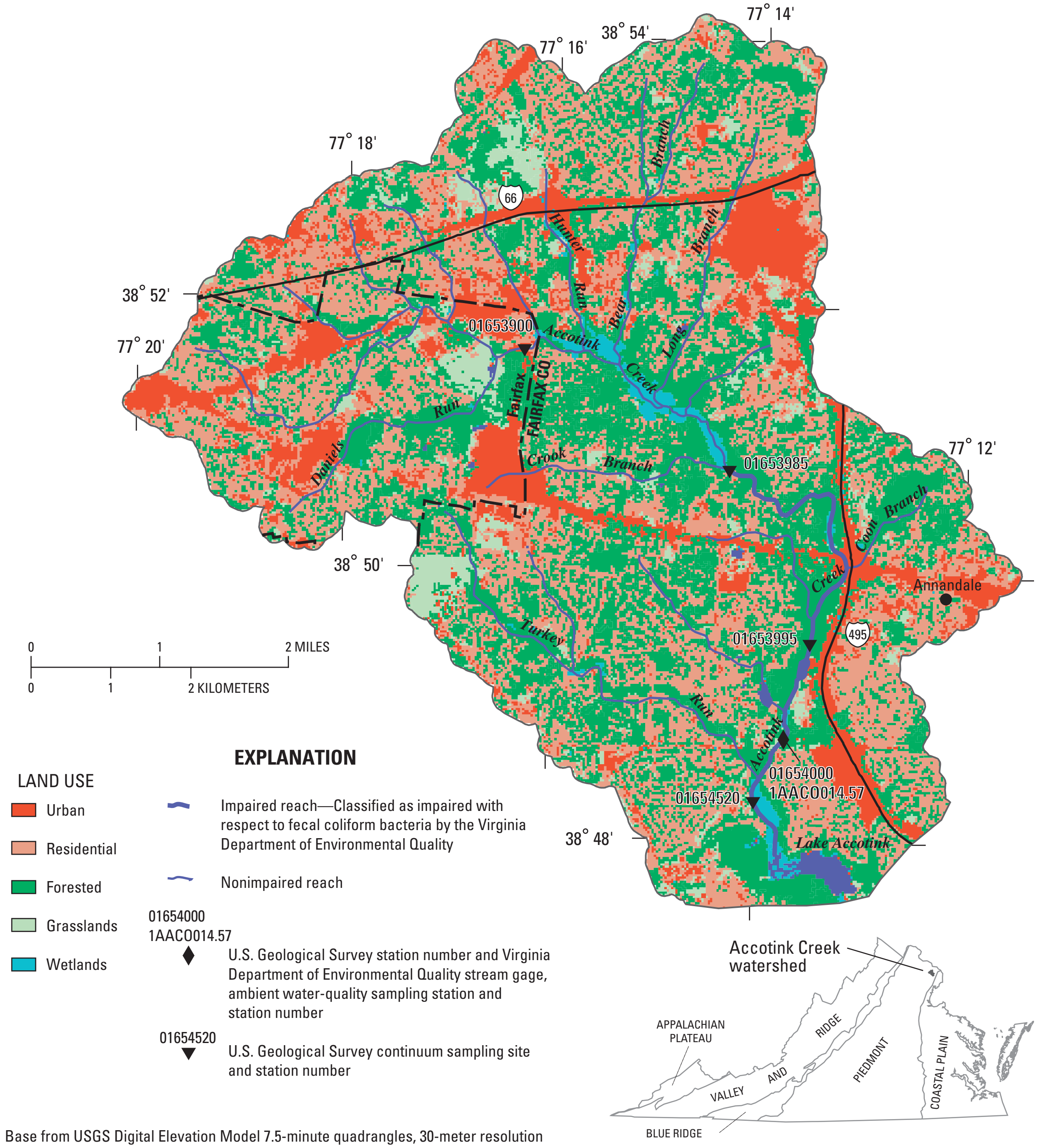
The soils of the Accotink Creek watershed are present as three distinct soil associations, described by Porter and others (1963). The Glenelg-Elioak-Manor association has developed from the weathering of the crystalline bedrock of the Piedmont. These well-drained (and, in some places, excessively drained) silt-loam soils dominate the watershed. The Fairfax-Beltsville-Glenelg association comprises a relatively small portion of the watershed (limited to the headwater areas) and formed from the residuum of Piedmont bedrock and fluvial Coastal Plain sediments. These soils are present as silt or sand loams, and range from somewhat poorly drained to well drained. The Chewacla-Wehadkee association occurs only on a limited basis within the watershed, generally in the bottomland and in floodplains along streams. These silt-loam soils range from moderately well drained to poorly drained and have developed from alluvial material that was washed from the Piedmont uplands.

Most water-quality data for this study were collected from the Accotink Creek stream gage (station number 01654000); this site also is a DEQ ambient water-quality sampling station. Four additional stations where data were collected (continuum sampling sites) along Accotink Creek are at Route 237 (Pickett Road, station number 01653900), Route 846 (Woodburn Road, station number 01653985), Woodlark Drive (station number 01653995), and Lonsdale Drive (station number 01654520).



Base from USGS Digital Line Graph, 1:2,000,000, 1987
County boundaries from 1:100,000, TIGER files, 1994
Universal Transverse Mercator 18 projection, NAD 27, central meridian 75°00'W

Figure 1. Location of Accotink Creek, Blacks Run, and Christians Creek watersheds, and physiographic provinces in Virginia.



Base from USGS Digital Elevation Model 7.5-minute quadrangles, 30-meter resolution

Land use from U.S. Environmental Protection Agency Multi-Resolution Land Characteristics Region 3, 1993, 30-meter resolution

Hydrography digitized from 1:24,000, 7.5-minute quadrangles

County boundaries from 1:100,000, TIGER files, 1994

Universal Transverse Mercator 18 projection, NAD 27, central meridian 75°00'W

Figure 2. Land use, streams, and sampling stations in the Accotink Creek watershed, Fairfax County, Virginia.

Christians Creek

Christians Creek, located in Augusta County, is the agricultural watershed selected for this study (fig. 3). Christians Creek originates northwest of Greenville, Va., and extends to the confluence with the Middle River. The entire 31.5-mi-long reach is classified as impaired with respect to fecal coliform bacteria. The watershed has a drainage area of 107 mi^2. The population of the watershed is estimated to be 12,000 (1990 U.S. Census Bureau data). There is a recently (1997) deactivated stream gage (still operational for instantaneous stage determinations) at Route 794 (Sangers Lane, station number 01624800), with a period of record from 1967 to 1997. DEQ has sampled for fecal coliform bacteria at Route 794 and Route 831 (Old White Hill Road, station number 1BCST021.76) on a monthly basis since 1991. The ambient water-quality sampling station at Route 794 was the primary Christians Creek sampling location for this study.

There are 18 permitted point source dischargers in the watershed (B.K. Fowler, Virginia Department of Environmental Quality, written commun., 2000; table 1). The Fishersville Sewage Treatment Plant discharges into Christians Creek about 1,500 ft upstream from one of the USGS and DEQ water-quality sampling locations (Route 794). On various occasions, the outfall from this sewage-treatment plant was sampled to check that it was not an important contributor of fecal coliform bacteria to the stream. As permitted, none of these point sources contributes greater than 200 col/100 mL to Christians Creek. None of these point sources represents a large flow contribution to Christians Creek; cumulatively, these sources account for less than 5 percent of the daily flow in the creek. The 12 private permitted dischargers in the watershed are 9 family residences and 3 small businesses.

Land use within the watershed is dominated by agricultural practices that are potential sources of fecal coliform bacteria within the watershed. Major components of animal husbandry in this watershed include the production of beef cattle, dairy cattle, heifers, broilers, and turkeys. Other potential fecal coliform bacteria sources within the watershed include humans (as contributed by failing septic systems, leaking or overflowing sewer lines, cross-pipes, and straight pipes), domestic animals (such as dogs and cats), waterfowl (such as geese, ducks, and sea gulls), and wildlife (such as deer, raccoons, opossum, rabbits, muskrats, ground hogs, foxes, and beaver).

The Christians Creek watershed lies within the Valley and Ridge physiographic province. The surficial geology that underlies the drainage basin is composed of 10 formations and is dominated by limestone and dolomite; information about each formation is summarized from Rader (1967). The Martinsburg Formation (calcareous shale and sandstone) is the dominant formation within the basin. Other formations in the watershed include the Edinburg Formation (argillaceous limestone and shale), Lincolnshire Formation (cherty limestone), New Market Limestone (limestone with dolomite beds near the base), Beekmantown Formation (dolomite and limestone), Chepultepec Formation (limestone and dolomite), Conococheague Formation

Table 1. Permitted point-source dischargers of fecal coliform bacteria in Christians Creek watershed during 2000, Augusta County, Virginia (B.K. Fowler, Virginia Department of Environmental Quality, written commun., 2000)

[Mgal/d, million gallons per day]

Discharger	Discharge (Mgal/d)	Latitude	Longitude
Fishersville Sewage Treatment Plant	0.7	38°07’41”	78°59’46”
Staunton Plaza Sewage Treatment Plant	.09	38°06’45”	79°03’18”
Brookwood Interchange Sewage Treatment Plant	.03	38°04’26”	79°04’56”
Riverheads High School Sewage Treatment Plant	.014	38°01’47”	79°08’27”
Southern States Cooperative	0	38°06’09”	79°04’24”
Woodlawn Village Mobile Home Park	.007	38°08’53”	78°55’06”
12 private permitted dischargers	.001	Various	Various

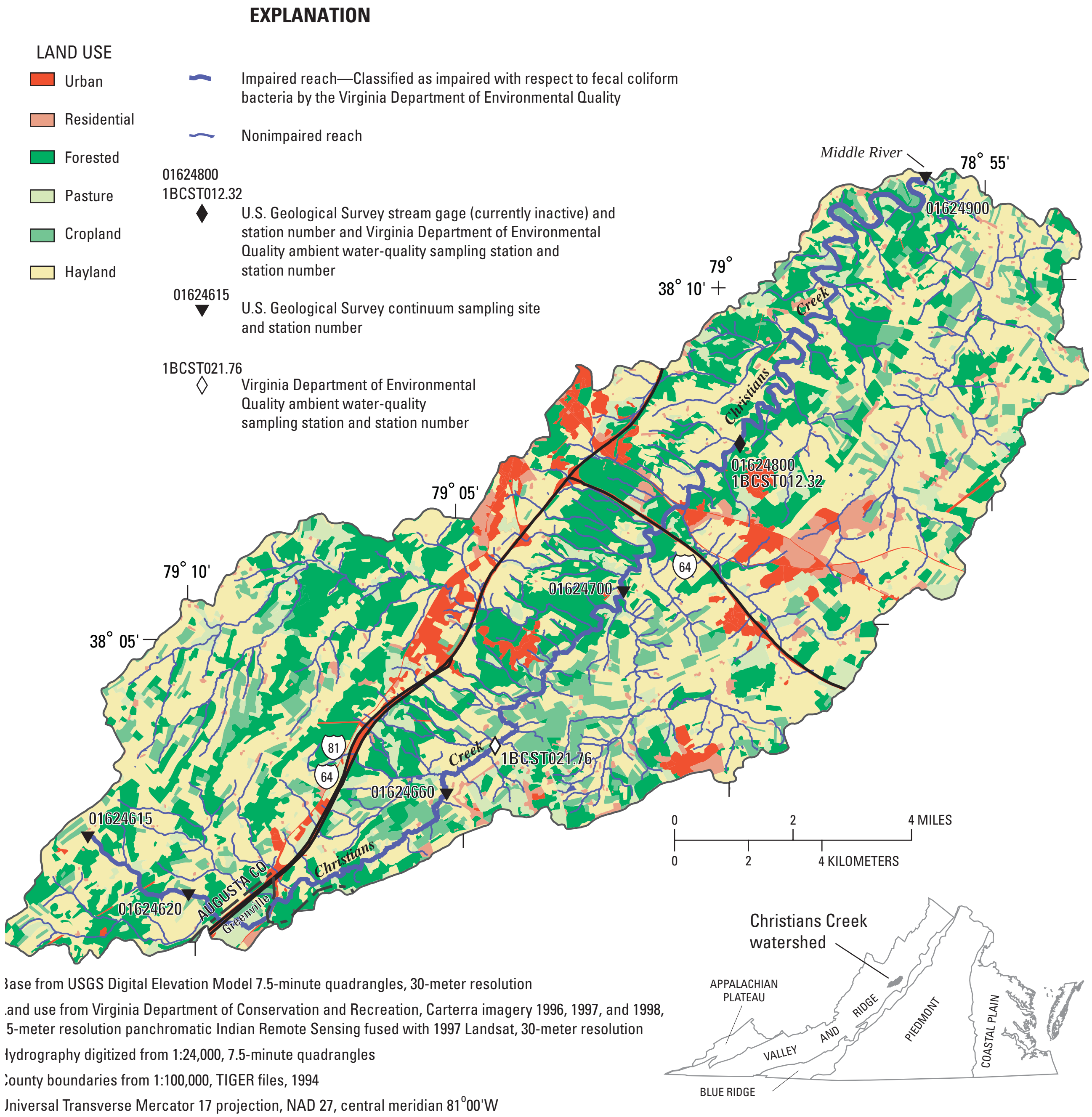


Figure 3. Land use, streams, and sampling stations in the Christians Creek watershed, Augusta County, Virginia.

(limestone, dolomite, and sandstone), and Elbrook Formation (limestone and dolomite). Alluvial material (composed of sand and clay) is present in portions of the floodplain adjacent to Christians Creek. Small amounts of fault breccia (large blocks of dolomite and limestone with crush conglomerate) also are present in the basin.

The soils of the Christians Creek watershed have been described thoroughly (Hockman and others, 1979) and are best classified as derived from the parent material from which they were formed. Much of the soil in the watershed has formed from the residuum of interbedded limestone, dolomite, and calcareous shale. Three soil assemblages have been identified in this category. The Frederick-Christian-Rock outcrop assemblage consists of deep, well-drained, silt loam or fine sandy loam soils with limestone outcrop areas. The Frederick-Bookwood-Christian assemblage consists of deep to moderately deep, well-drained, silt loam or fine sandy loam soils; scattered sinkholes or rock outcrops also may be present. The Chilhowie-Edom assemblage consists of deep to moderately deep, well-drained, silt loam or silty clay loam soils with occasional bedrock outcrops. Soil also has formed from the residuum of shale and thin interbedded sandstone and limestone. These soils are a part of the Berks-Weikert-Sequoia assemblage, which consists of shallow to deep, well-drained, silt loam or shaly silt loam soils. On floodplains and terraces, soils have formed in the alluvial or colluvial material. Although not extensive within the watershed, these soils are part of the Buchanan-Wheeling-Buckton assemblage, which consists of deep, somewhat poorly drained to well-drained soils. Generally these soils consist of silt loam, loam, or fine sandy loam, although some soils are gravelly or cobbly.

Most water-quality data were collected from Christians Creek below the bridge at Route 794 (Sangers Lane, station number 01624800); this site also is a DEQ ambient water-quality sampling station. Five additional sampling stations (continuum sampling sites) along Christians Creek were at the spring near Route 693 (Berry Moore Road, station number 01624615), Route 604 (McClures Mill Road, station number 01624620), Route 340 (Stuarts Draft Highway, station number 01624660), Route 635 (Barterbrook Road, station number 01624700), and Route 612 (Laurel Hill Road, station number 01624900).

Blacks Run

Blacks Run, located in Rockingham County, is the mixed urban and agricultural watershed selected for this study (fig. 4). Blacks Run originates on the north side of the city of Harrisonburg and extends to the confluence of Cooks Creek. The entire 10.7-mi-long reach is classified as impaired with respect to fecal coliform bacteria. The watershed has a drainage area of 20 mi^2 and an estimated population of 34,700 (1990 U.S. Census Bureau data). The city of Harrisonburg is the primary urban area within the watershed. This stream, like many in Virginia, did not have a stream gage, so one was installed (station number 01621470) at Route 704 (Cecil Wampler Road) in 1999. DEQ has sampled for fecal coliform bacteria at this station on a monthly basis since 1991.

There are no sewage-treatment plants in the Blacks Run watershed, but there are two private permitted dischargers, one family residence and one small business (B.K. Fowler, Virginia Department of Environmental Quality, written commun., 2000). Under the discharge permits, the treated wastewater discharge may not exceed 1,000 gallons per day and may not contain fecal coliform bacteria concentrations that exceed 200 col/100 mL.

Approximately two-thirds of the watershed (generally the portion closer to the headwaters) is dominated by urban land uses. In this urban area, potentially major contributors of fecal coliform bacteria include humans (as contributed by cross-pipes, failing septic systems, and leaking or overflowing sewer lines), domestic animals (such as dogs and cats), waterfowl (such as geese, ducks, and sea gulls), and wildlife (such as raccoons, opossum, rats, squirrels, and deer). The remaining one-third of the watershed (the lower portion of the watershed, closer to the stream gage) is dominated by agricultural land uses. Major components of the animal husbandry in this watershed include the production of beef cattle, dairy cattle, heifers, chickens, broilers, and turkeys. Other potential contributors in this agricultural area include humans (as contributed by failing septic systems, leaking or overflowing sewer lines, cross-pipes, and straight pipes), domestic animals (such as dogs and cats), waterfowl (such as geese, ducks, and sea gulls), and wildlife (such as deer, raccoons, opossum, rabbits, muskrats, ground hogs, foxes, and beaver).

The Blacks Run watershed lies within the Valley and Ridge physiographic province. The surficial geol

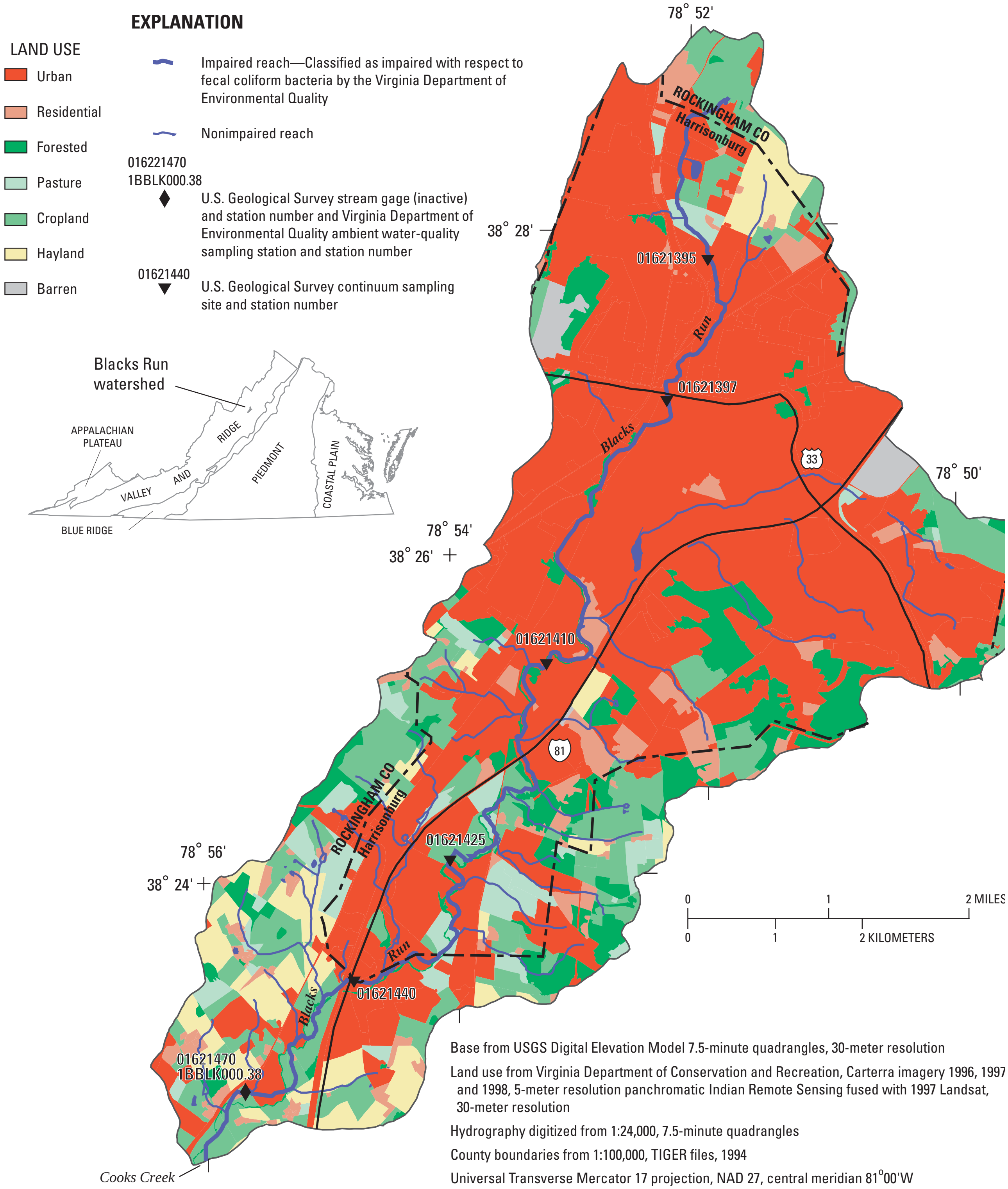


Figure 4. Land use, streams, and sampling stations in the Blacks Run watershed, Rockingham County, Virginia. Streams that appear disconnected are continuous; however, development activities within the watershed have captured these streams and routed the streamflow under portions of the city of Harrisonburg. Barren areas are primarily quarries.

ogy of the watershed is composed of seven formations and is dominated by limestone and dolomite; information about each formation is summarized in Gathright and Frischmann (1986). The primary formations within the watershed include the Martinsburg Formation (calcareous slate, argillite, and sandstone), Beekmantown Group (limestone and dolomite), New Market Limestone (limestone with dolomite beds near the base), Lincolnshire Formation (cherty limestone), Oranda Formation (limestone and calcareous shale), and Edinburg Formation (limestone and calcareous shale). Karst features are evident in portions of the watershed. Alluvial material (composed of unconsolidated fine sand, silt, and minor clay) is present in portions of the floodplain adjacent to Blacks Run.

The soils of the Blacks Run watershed have been described thoroughly (Hockman and others, 1982) and are best classified as derived from the parent material from which they were formed. Most of the soil in the watershed has formed from the residuum of limestone, dolomite, and calcareous shale. Three soil assemblages have been identified in this category. The Frederick-Lodi-Rock outcrop assemblage consists of deep, well-drained, silt loam soils with limestone or dolomite outcrop areas. The Endcav-Carbo-Rock outcrop assemblage consists of deep and moderately deep, well-drained, silt loam soils; sinkholes and limestone outcrops are common in this assemblage. The Chilhowie-Edom assemblage consists of deep to moderately deep, well-drained, silt loam or silty clay loam soils with occasional bedrock outcrops. On floodplains and terraces, soils have formed in the alluvial or colluvial material. Although not extensive within the watershed, these soils are part of the Monongahela-Unison-Cotaco assemblage, which consists of deep, well-drained or moderately well drained soils. Generally these soils consist of fine sandy loam soils, although some soils are cobbly.

Most water-quality data for this study were collected from Blacks Run below the bridge at Route 704 (Cecil Wampler Road, station number 01621470); this site also is a DEQ ambient water-quality sampling station. Five additional sampling stations (continuum sampling sites) along Blacks Run were at Route 753 (Liberty Street, station number 01621395), Water Street (station number 01621397), Route 726 (Stone Spring Road, station number 01621410), Route 679 (Pleasant Valley Road, station number 01621425), and Route 988 (station number 01621440).

METHODS

Water-sample collection for bacteria

Intensive streamwater sampling at the ambient water-quality sampling station of each watershed was done to provide an understanding of the temporal patterns in fecal coliform concentrations and the specific sources of these bacteria at each sampling site. Streamwater samples were collected over a wide range of hydrological conditions. Low-flow samples were collected from each stream approximately every 6 weeks, and approximately 4 of these low-flow samplings in each watershed were performed on the recession limbs of storm events. Typically, between four and eight depth-integrated samples were collected at each sampling site during each low-flow sampling. Width integration was accomplished by sampling at three locations across the width of the stream (the center of the channel and approximately halfway to each stream bank). The depth-integrated samples were collected at 5-minute intervals, providing time integration during each sampling. Five storm events were sampled on each stream. During each storm event, at least 10 water samples were collected from approximately the center of the streamflow. When possible, the storm samples were collected such that the first three samples were collected on the rising limb of the hydrograph, the next four samples were collected around the peak in the hydrograph, and the last three samples were collected on the falling limb of the hydrograph (fig. 5).
All samples were collected using sterile, 160-ml, narrow-mouth, borosilicate glass bottles. The samples were collected from the stream using the hand-dip method or a weighted-bottle sampler, depending on the site and flow conditions. Samples were immediately chilled on ice and processed in the field within 6 hours of collection.

Continuum sampling sites were established at 2- to 4-mi intervals along each of the three stream reaches, resulting in a total of four or five continuum sites on each reach. These continuum sites were sampled at various times during this study to evaluate whether the intensive sampling at the ambient water-quality sampling station represented the entire watershed. Each continuum sample was collected as a single, depth-integrated sample from the approximate center of the streamflow.

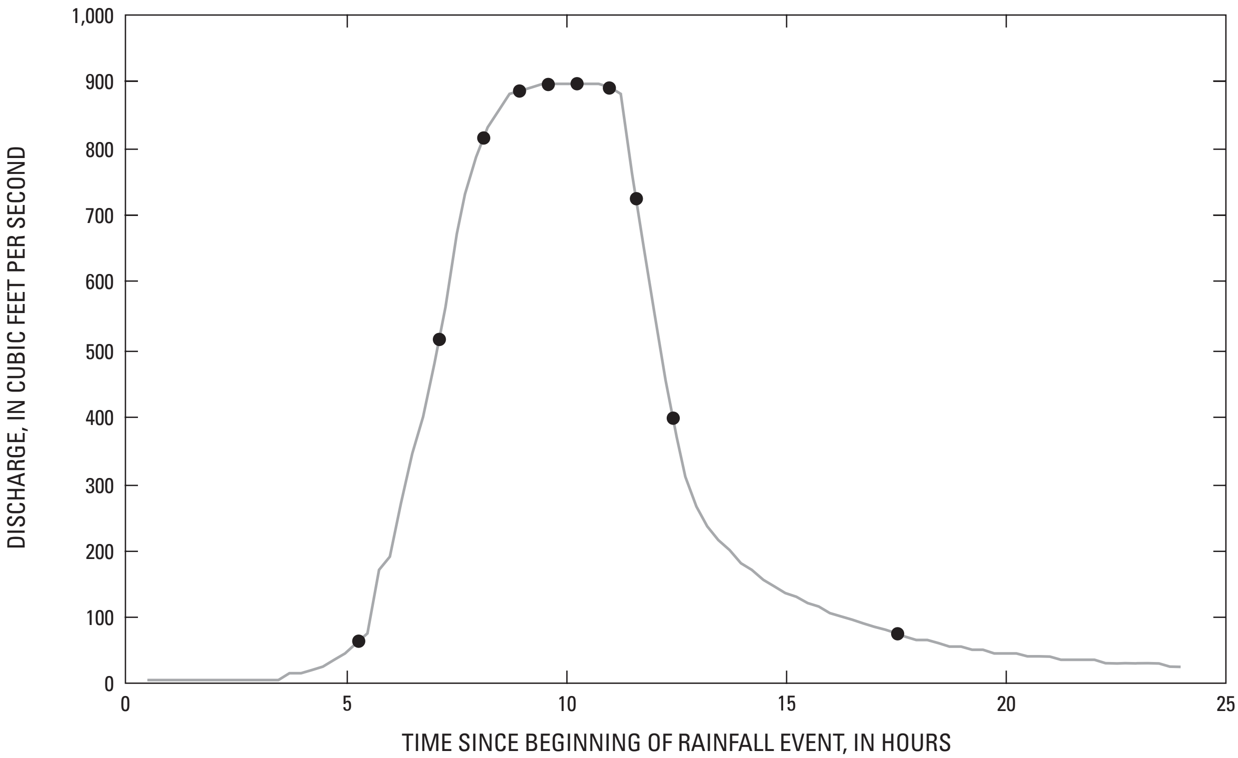


Figure 5. Storm-flow sampling design for bacterial source tracking study in Accotink Creek, Blacks Run, and Christians Creek watersheds, Virginia.

Synoptic samples of Accotink Creek were collected on June 5, 2000, following a major storm event. Various storm drains, major stream tributaries, and main channel sites were sampled to determine whether the entire watershed was contributing fecal coliform bacteria to the stream. Rhodamine WT dye was injected into the stream headwaters, and synoptic samples were collected while moving downstream at a rate that was consistent with the stream velocity and the injected dye. A single water sample (a grab sample) from the approximate center of the streamflow was collected from each sampling site. During this synoptic survey, a consistent water parcel was sampled as it traveled from the headwaters to the stream gage.

Supporting field measurements

Streamwater discharge and field water-quality parameters (pH, turbidity, dissolved oxygen concentration, water temperature, and specific conductance) were measured during the collection of each of the water samples for bacteria enumeration. Discharge measurements were made following standard USGS methods (Rantz and others, 1982). All field parameters were determined in accordance with the standard methods of the USGS (Wilde and Radke, 1998). The pH, water temperature, and specific conductance were measured using a YSI Model 63 handheld field meter. The dissolved oxygen concentration was measured using a YSI Model 95 handheld field meter. Turbidity was determined using a HACH 2100P handheld portable turbidimeter. All meters were calibrated (or quality assured, as appropriate) at the start of each field day, in accordance with the manufacturers' instructions. Specific conductance, dissolved oxygen concentration, pH, and water temperature were measured in situ by positioning the probes in the center (or as close as possible to the center) of the streamflow. Turbidity was measured on aliquots obtained from the water samples that were processed for bacteria.

Fecal coliform enumeration

All samples for the enumeration of fecal coliform bacteria were collected and processed according to USGS standard methods (Myers and Sylvester, 1997).

Water samples were processed in the field by membrane filtration (using gridded, 0.7-µm pore size membrane filters), and filters were incubated on a media of m-FC broth. Through this technique, fecal coliform bacteria are defined operationally as organisms that produce blue colonies in whole or in part after incubation for 18 to 22 hours at 44.5 ± 0.2ºC. A range of sample dilutions was always prepared in an effort to have at least one filter with colonies in the ideal counting range (20-60 colonies). The filter apparatus, bench tops, and necessary equipment were sterilized between the processing of each water sample. Start and end sample blanks were processed to ensure that the equipment initially was sterile, and that between-dilution rinsing procedures were adequate. Replicates were processed on 6 percent of the samples. After incubation, fecal coliform colonies were counted and the concentration of bacteria in the streamwater sample was calculated (as col/100 mL) based on the volume of filtered sample.

E. coli enumeration

About 150 fecal coliform samples (approximately 50 from each watershed) also were enumerated for *Escherichia coli* (*E. coli*) concentrations. *E. coli* were enumerated following standard USGS methods (Myers and Sylvester, 1997). Water samples were processed in the field by membrane filtration (using gridded, 0.45-µm pore size membrane filters), and filters were incubated on m-TEC agar. Through this technique, *E. coli* bacteria are defined operationally as organisms that produce yellow or yellow-brown colonies after resuscitation at 35.0 ± 0.5ºC for 2 hours and incubation for 22 to 24 hours at 44.5 ± 0.2ºC. A range of sample dilutions was always prepared in an effort to have at least one filter with colonies in the ideal counting range (20-80 colonies). The filter apparatus, bench tops, and necessary equipment were sterilized between the processing of each water sample. Start and end sample blanks were processed to ensure that the equipment initially was sterile, and that between-dilution rinsing procedures were adequate. After incubation, *E. coli* colonies were counted and the concentration of the streamwater sample was calculated (as col/100 mL) based on the volume of filtered sample.

A paired comparison of fecal coliform and *E. coli* concentrations was performed to verify that *E. coli* were the primary component of the fecal coliform bacteria that were observed in the streams. This verification was important because the ribotyping was performed on *E. coli*, and the Commonwealth of Virginia determined the water quality of streams and rivers on the basis of a fecal coliform standard. A strong correlation is present between fecal coliform and *E. coli* concentrations (fig. 6); most of the fecal coliforms collected in these three streams (67 percent) were *E. coli*. These results justify the use of *E. coli* bacteria for ribotyping even though the water-quality standard is based on fecal coliform bacteria.

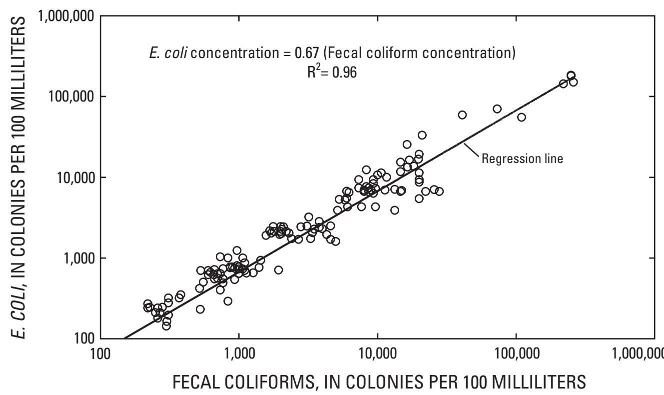


Figure 6. Relation of fecal coliform and *E. coli* concentrations in water samples collected March 1999 through October 2000 in the Accotink Creek, Blacks Run, and Christians Creek watersheds, Virginia.

Bacterial source tracking

Ribotyping was selected as the BST technique for this study because it offered definitive source identification and produced results that should be applicable to detailed TMDL development. Dr. Mansour Samadpour's Microbial Source Tracking Laboratory at the University of Washington (UWMSTL) performed the bacterial source tracking for all samples in this study. Although the specific application to field-based source identification is relatively new, ribotyping is a well-established tool in molecular biology (Tarkka and others, 1994; Schalch and others, 1997; Dalla-Costa and others, 1998; Samadpour, 2001). Conceptually, ribotyping is successful for this application because individual *E. coli* strains generally are host-species specific—only infrequently does an *E. coli* strain colonize a foreign host species. Subtle genetic differences are present among *E. coli* strains, and ribotyping is able to characterize these differences. After isolating and characterizing an *E. coli* strain from an unknown source, the strain is compared with a known source database (developed from the feces of potential source animals) to identify the source of the *E. coli*. The ribotyping technique makes use of the portion of the *E. coli* genome that codes for the production of ribosomal RNA (ribonucleic acid). This portion of the *E. coli* genome is believed to be stable and intolerant of genetic mutations. Consequently, individual *E. coli* strains should maintain the same genes for ribosomal RNA production over many generations, and the occurrence of each *E. coli* strain can be tracked over extended time periods.

Standard microbiological and molecular biology techniques were used in the ribotyping analysis. The following is a brief description of the steps used in the ribotyping procedure:

1. *Isolation of E. coli bacteria*: For each water sample, a single fecal coliform plate was sent to the UWMSTL, where it was logged into the tracking system. Between 3 and 5 *E. coli* colonies were isolated from each fecal coliform plate. Colonies were cultured on MacConkey Agar following standard techniques and confirmed using biochemical tests (indole production from tryptophane, and lack of growth on a citrate media).
2. *Preservation of pure cultures*: Isolated *E. coli* colonies were stored by freezing at –80ºC in a nutrient broth that contained 15-percent glycerol.
3. *Isolation of genetic material*: Isolated *E. coli* colonies also were cultured on a nutrient medium for isolation of their genetic material. Cells were collected from the nutrient medium and lysed (broken open). After various cleanup and extraction steps, the free DNA material was isolated from the remainder of the cellular material.
4. *Digestion of the DNA material using restriction enzymes*: The isolated DNA material was digested (cut into fragments of variable length that depended on the specific base sequence that the enzyme recognized) in separate reactions using a pair of restriction enzymes (*Eco*RI and *Pvu*II). Each enzyme produces a different, but highly specific digestion of the DNA.
5. *Gel electrophoresis to separate the digested DNA material*: The DNA fragments were loaded into an agarose gel and an electrical field was applied to the gel. Because the DNA fragments are negatively charged, the induced current causes them to migrate away from the negative electrode; the agarose gel is sufficiently permeable that the small DNA fragments migrate faster than the larger fragments. After 17 hours, the DNA fragments become separated according to the size of the fragment and the current is discontinued. One specific *E. coli* isolate (labeled isolate #3915) was included with every gel to allow size comparisons among individual gels. Following electrophoresis, the DNA fragments in the gel were stained with ethidium bromide (which fluoresces under an ultraviolet light source), and the gel was placed under an ultraviolet light to ensure that complete digestion occurred and that the electrophoresis was successful. If digestion and electrophoresis are successful, a fluorescent band of DNA will generally extend from the lower edge of the gel (where the DNA fragments were initially loaded) to the upper edge of the gel (near the positive electrode).
6. *Transfer of the DNA fragments from the agarose gel onto a nylon membrane*: Once electrophoresis was completed, the DNA material was manipulated further before being transferred onto a nylon membrane. First, the DNA fragments were cut up further (using hydrochloric acid) to allow an easier transfer from the gel onto the paper. Second, the DNA was denatured (using sodium hydroxide) into single strands to allow recombination with the gene probe. Neither of these treatments affected the positioning of the DNA within the gel. After these two manipulations, the single-stranded DNA fragments were transferred from the agarose gel onto the nylon membrane. This procedure is known as the Southern blot procedure. After the transfer was complete, the nylon membrane was air dried and baked to fix the single-stranded DNA fragments to the membrane.
7. *Hybridization with the radiolabeled cDNA probe:* The radiolabeled cDNA probe was prepared by extension of random hexanucleotide primers. The probe and the nylon membrane then were combined in a hybridization solution, and the probe was given time to hybridize, or bind with any complementary, single-stranded DNA fragments on the nylon membrane. Following hybridization, the nylon membrane was washed to remove any non-specific binding of probe material and then allowed to dry. Only regions containing single-stranded DNA complementary to the cDNA probe retained the radioactive label.

8. *Generation of the autoradiograph:* The dry membranes were exposed to X-ray films; the hybridized regions appear as dark bands on the radiograph (fig. 7). This specific banding pattern is the "ribotype" for a particular *E. coli* isolate.
9. *Comparison of the unknown E. coli banding pattern to the known-source library of patterns*: The unknown ribotype was compared to the known-source library (described below) to see if the unknown pattern matched a source that was already sampled. Analysis and identification of the unknown isolate banding pattern was performed by assigning a numerical value to each ribotype based on the distance between bands. Bands that were more than 3 mm apart were counted as single bands, whereas bands that were within 3 mm of each other were counted as double or triple bands (for example, two bands that were closer than 3 mm to each other were designated a "2" and three bands with 3 mm or less between each band were designated a "3." In this manner, each banding pattern was assigned a specific numeric value. Two isolates with the same numeric value but different banding patterns (because the actual bands may be shifted and not identical) were assigned letters to differentiate the two ribotypes; for example, 2122111A and 2122111B would identify two isolates with similar but slightly offset banding patterns. Isolates with the same numeric values for their ribotypes were deemed to be members of the same ribogroup. The known-source library of ribotype patterns was stored in an electronic database that also included information on the animal source from which each known isolate was obtained. Unknown isolates were queried against this database, based on the numeric value. If an unknown isolate had the same numeric value as any in the known-source library, the unknown ribotype was compared directly to all the known-source isolates that were members of this particular ribogroup. The unknown isolate was identified only if the banding pattern of the unknown isolate visually matched an isolate in the library for both the restriction enzymes. Any unknown isolate that did not match a sample in the known source library was labeled "unknown."

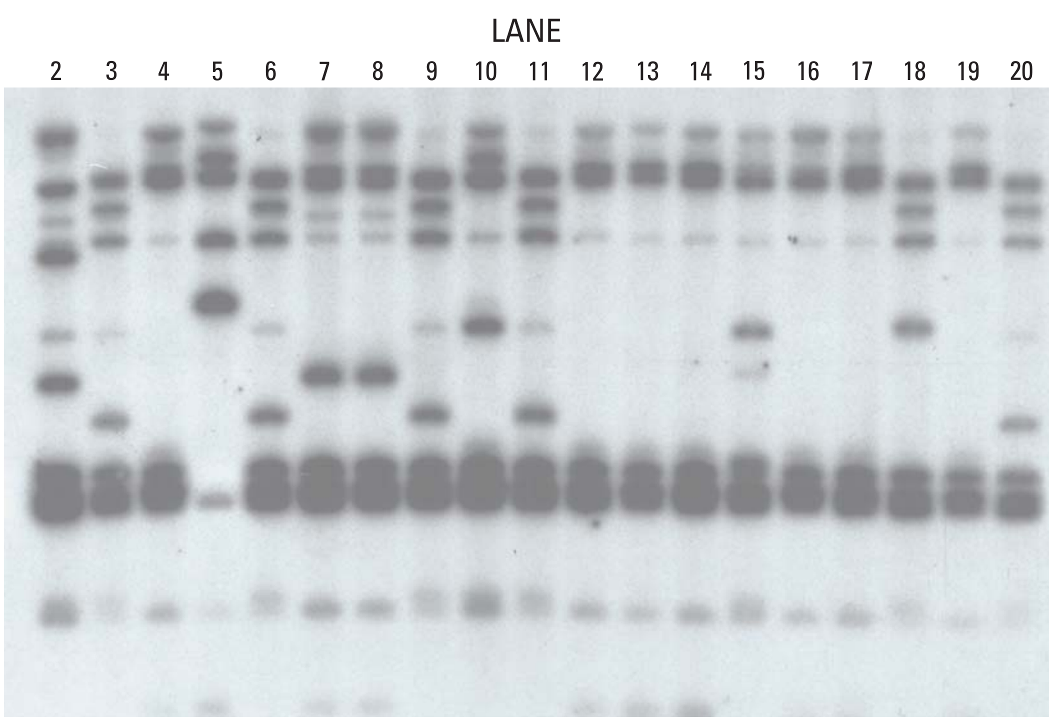


Figure 7. Example of the banding patterns produced by the ribotyping procedure. Each lane represents the pattern generated by a single *E. coli* isolate.

Source-sample collection and source library development

A source-sample library is necessary for a successful source tracking study. The source-sample library provides a set of known ribotype patterns with which the unknown isolates can be compared and identified. The extensive source library at the UWMSTL contained approximately 50,000 isolates. In addition, a site-specific source library was developed for this study by collecting known-source fecal samples from most of the potentially contributing animal sources in each of the three watersheds studied. Fresh fecal samples (of known origin) were collected from farms, animal shelters, veterinary clinics, animal rehabilitation centers, sewage-treatment plants, forested areas, and public parks. These fecal samples were collected aseptically, placed in sterile specimen containers, labeled by source, and sent by overnight delivery to the UWMSTL. At the laboratory, a single *E. coli* isolate was cultured from each fecal sample, ribotyped, and added to the source-library database.

PATTERNS AND SOURCES OF FECAL COLIFORM BACTERIA

Overview of the water samples collected

A total of 605 water samples was collected from the three study streams during this investigation. The distribution of the total number and the type of water samples collected are presented in table 2. Approximately two-thirds of the samples from the ambient water-quality sampling stations in each watershed were collected during low-flow conditions; the remaining one-third of all samples were collected during storm-flow periods. The collection of water samples during both low-flow and storm-flow periods is critical for accurately describing both bacteria concentrations and bacteria sources in a surface-water system. Continuum samples were also collected to investigate the spatial patterns of the fecal coliform concentrations along the length of each study stream.

Fecal coliform analyses

Spatial patterns in the fecal coliform concentrations

The continuum streamwater samples provided evidence that the fecal coliform concentrations observed at the ambient water-quality sampling station of each stream were reflective of the water-quality conditions for the entire stream (table 3). Although concentrations of fecal coliforms were variable among continuum sites on a given day, the streamwater quality (relative to the water-quality standard) generally was consistent among sites; if the water-quality standard was violated at the ambient water-quality sampling station, then the standard typically was violated at the other continuum sampling sites on that day. Similarly, if the water-quality standard was met at the ambient water-quality sampling station, then the other continuum sampling sites also were generally in compliance with the standard. Several of the continuum samples had extremely elevated fecal coliform concentrations (Accotink Creek on June 6 and August 8, 2000; Blacks Run on March 22, July 22, September 5, and October 4, 1999). All six of these sampling events were performed under storm-affected flow conditions (there had been appreciable rainfall within the past 48 hours and the flow was still receding). These storm-affected samples provided evidence that the fecal coliform concentrations increase during storm-flow periods. Another Accotink Creek sampling event on August 11, 1999, is of interest because it occurred during extended drought conditions and the stream had been reduced to a series of disconnected pools; the samples from these disconnected

Table 2. Number and type of streamwater samples collected from March 1999 through October 2000 in three watersheds in Virginia

Watershed	Number of samples collected			
	Low flow	Storm flow	Continuum	Total
Accotink Creek	104	53	36	193
Christians Creek	104	66	18	188
Blacks Run	99	56	69	224

Table 3. Fecal coliform concentrations of the continuum samples in three watersheds in Virginia, 1999-2000

[Location of stations on figures 2-4; col/100 mL, colonies per 100 milliliters; –, no sample collected]

Accotink Creek watershed

Continuum sample number	Station number	Fecal coliform concentration (col/100mL)			
		Sampling date			
		3/18/99	8/11/99	6/6/00[a]	8/8/00[a]
1	01653900	320	190	38,000	13,000
2	01653985	200	25	18,000	15,000
3	01653995	50	54	23,000	17,000
4	01654000[b]	73	37	13,000	13,000
5	01654520	64	42	–	9,300

Christians Creek watershed

Continuum sample number	Station number	Fecal coliform concentration (col/100mL)		
		Sampling date		
		3/25/99	7/27/99	8/1/00
1	01624615	5	71	7
2	01624620	87	1,500	300
3	01624660	230	2,000	3,800
4	01624700	23	6,400	1,900
5	01624800[b]	15	790	1,800
6	01624900	9	140	830

Blacks Run watershed

Continuum sample number	Station number	Fecal coliform concentration (col/100mL)												
		Sampling date												
		3/22/99[a]	7/22/99[a]	8/19/99	9/5/99[a]	10/4/99[a]	11/17/99	12/17/99	1/22/00	2/25/00	3/28/00	4/27/00	5/13/00	8/15/00
1	01621395	290,000	–	–	86,000	2,300	–	–	–	16	200	450	–	–
2	01621397	–	23,000	760	62,000	4,600	300	1,100	20,000	120	770	340	2,000	6,000
3	01621410	100	54,000	610	41,000	4,300	69	160	–	26	42	170	770	520
4	01621425	400	81,000	820	22,000	2,300	33	110	13	80	130	610	1,700	2,000
5	01621440	2,000	39,000	94	21,000	1,100	10	18	10	13	160	73	200	380
6	01621470[b]	2,200	7,200	22,000	65,000	6,300	410	6,000	20	390	180	61	580	700

[a] Storm-affected sample (rain had occurred in the last 48 hours and flow was receding)
[b] Sampling station is co-located with a stream gage

pools had some of the lowest fecal coliform concentrations that were observed during the study.

The synoptic sampling of Accotink Creek on June 6, 2000, provided further evidence that the entire watershed was contributing fecal coliforms (table 4). This synoptic sampling was performed immediately following a storm event and included samples from various storm drains, major stream tributaries, and main channel sites (all samples were collected while moving in the downstream direction). All sampled storm drains and stream tributaries had elevated concentrations of fecal coliform bacteria, and all samples collected during this synoptic survey exceeded the Commonwealth's instantaneous water-quality standard for fecal coliform bacteria (1,000 col/100 mL). However, because these samples were collected at different times and on different portions of the storm recession (while the water quality of the entire stream system was changing rapidly), direct comparisons of fecal coliform concentrations among sites would not be meaningful. For example, these data do not support the conclusion that the fecal coliform contributions from Daniels Run were greater than the contributions from Coon Branch because these two samples were collected approximately 9 hours apart and on different portions of the hydrograph. Rather, one could conclude that elevated concentrations of fecal coliform bacteria occurred throughout the watershed and that all areas sampled contributed to these elevated concentrations.

Temporal patterns in the fecal coliform concentrations

Seasonal patterns were evaluated in the fecal coliform concentrations at each ambient water-quality sampling station. Water samples were collected over a 20-month period, with 15 sampling events at each site during low-flow periods. Between four and eight water samples were collected during each low-flow sampling event, and these low-flow fecal coliform concentrations are summarized (fig. 8). For some of the low-flow sampling events, stream discharge records and meteorological data indicated that the streamflow was receding and that rain had fallen within the last 48 hours. These recession-flow samples (identified in figure 8) represent a subset of the low-flow samples. Most of the low-flow samples, however, were not collected under periods of recession flow, and this other subset of low-flow samples is referred to as base-flow samples.

In Accotink Creek, base-flow fecal coliform concentrations were generally below the instantaneous water-quality standard of 1,000 col/100 mL (fig. 8); however, the recession-flow samples occasionally exceeded the water-quality standard. The recession-flow fecal coliform concentrations were significantly elevated relative to the base-flow fecal

Table 4. Fecal coliform concentrations of water samples collected in the Accotink Creek watershed, Virginia, during synoptic sampling, June 6, 2000

[col/100 mL, colonies per 100 milliliters; samples collected and stations listed in downstream order; storm-drain station numbers increase in the downstream direction]

Main channel station	Fecal coliform (col/100mL)	Stream tributary sampling station	Fecal coliform[a] (col/100mL)	Storm-drain sampling station number	Fecal coliform (col/100mL)
Above Daniels Run	33,000	Daniels Run	100,000	1	21,000
01653900	38,000	Hunters Run	22,000	2	31,000
01653985	18,000	Bear Branch	22,000	3	10,000
01653995	23,000	Long Branch	30,000	4	10,000
01654000	13,000	Crook Branch	18,000	5	2,000
		Coon Branch	13,000		
		Turkey Run	1,100		

[a] Upstream sites were sampled immediately following a storm; more downstream sites were sampled later on the recession curve of the storm hydrograph

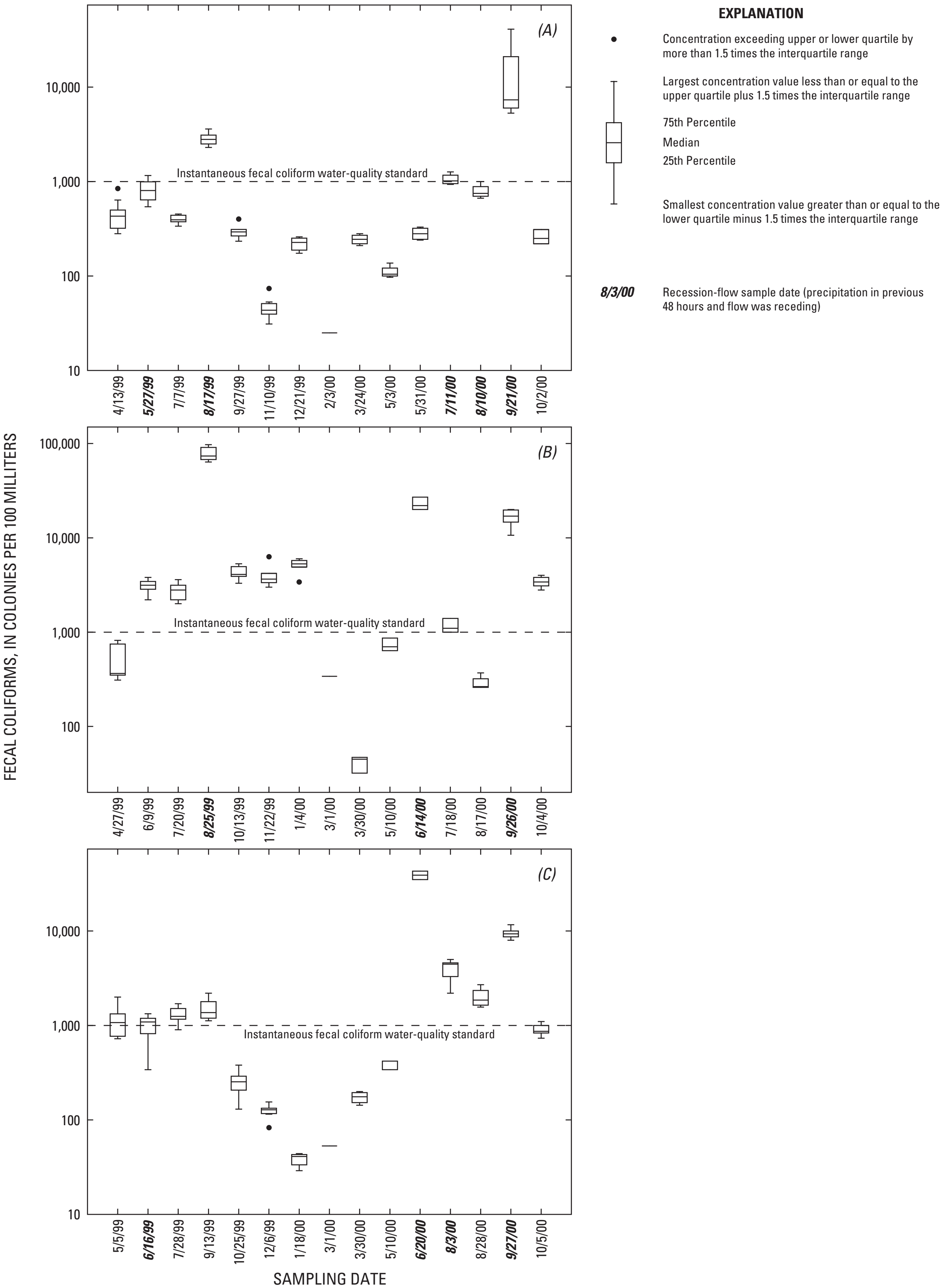


Figure 8. Fecal coliform concentrations during low-flow sampling of Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)* watersheds, Virginia, 1999-2000.

coliform concentrations ($p < 0.05$ using a Wilcoxon rank-sum test). The timing of the collection of some of the recession-flow samples makes a seasonal evaluation difficult; however, there appeared to be a slight seasonality with slightly lower fecal coliform concentrations during the winter and slightly higher concentrations during the warmer months. Pronounced seasonality in the fecal coliform concentrations was not expected in Accotink Creek because the land-use practices and potential fecal coliform sources in the watershed can be considered constant throughout the year.

Low-flow fecal coliform concentrations in Blacks Run were elevated relative to the Commonwealth's water-quality standard (fig. 8). Similar to Accotink Creek, the recession-flow water samples had fecal coliform concentrations that were significantly higher than those observed during base-flow conditions ($p < 0.05$ using a Wilcoxon rank-sum test). More than half of the base-flow water samples had fecal coliform concentrations that exceeded the 1,000 col/100 mL fecal coliform standard. A seasonal pattern was present that was similar to, but more pronounced than the one observed in Accotink Creek. The highest base-flow fecal coliform concentrations occurred during the summer and into the fall. During the winter, fecal coliform concentrations decreased to a minimum and then increased during the spring. This seasonal pattern is consistent with the animal management practices in the watershed. Livestock numbers typically are greatest during the summer and fall, and during these warm months the animals (particularly cattle) spend more time closer to and sometimes wading into the stream. This increased association of animals with the stream likely results in both direct deposition of feces into the stream and deposition of feces closer to the stream than during other times of the year. In addition to animal management practices, it also is possible that seasonally different fecal coliform survival rates (greater bacteria survival during the warm summer, relative to the cold winter, for example) may have affected these observed fecal coliform concentrations and contributed to the observed seasonal patterns.

Low-flow fecal coliform concentrations in Christians Creek also demonstrated a seasonal pattern (fig. 8). Approximately half of the base-flow water samples had fecal coliform concentrations that exceeded the Commonwealth's water-quality standard. Recession-flow samples had fecal coliform concentrations that were significantly elevated relative to the base-flow samples ($p < 0.05$ using a Wilcoxon rank-sum test). The seasonal variation in the Christians Creek fecal coliform concentrations was more pronounced and followed the same pattern as at Blacks Run and Accotink Creek. The highest base-flow fecal coliform concentrations occurred during the warm summer months, concentrations decreased through the fall, reached a minimum during the winter, and then concentrations increased through the spring. This pattern again is consistent with the animal practices in the watershed (increased animal density and activity around the streams during the hot summer months) and possible seasonal differences in survival rates of fecal coliform bacteria. Similar seasonal patterns have been described in other studies of fecal coliform concentrations and loads (Christensen and others, 2001; Baxter-Potter and Gilliland, 1988).

Fecal coliform concentrations were also monitored during five storm events on each study stream. At least 10 water samples were collected during each storm event, and as possible, the entire storm hydrograph (rising limb, plateau, and falling limb) was sampled. The fecal coliform concentrations observed during these storm events (fig. 9) were significantly elevated ($p < 0.05$ using Wilcoxon rank-sum test) relative to the observed base-flow fecal coliform concentrations (the recession-flow samples were not included for this analysis), and the water-quality standard was usually exceeded during these storm events. A large range of concentrations was observed during the individual storms because of the comprehensive sampling over the entire hydrograph. Peak fecal coliform concentrations observed during storm events on each study stream were 340,000 col/100 mL in Accotink Creek; 260,000 col/100 mL in Blacks Run; and 730,000 col/100 mL in Christians Creek. These elevated fecal coliform concentrations during storm events were anticipated on the basis of the results of previous studies (Christensen and others, 2001; Bolstad and Swank, 1997). In other studies, these elevated storm-flow concentrations have been interpreted as a combination of a flushing response (whereby fecal coliform bacteria that were deposited near the stream are washed off the land surface and into the stream) and a re-suspension of streambed sediments containing fecal coliform bacteria (Hunter and others, 1992; McDonald and Kay, 1981). Similar mechanisms likely were responsible for the storm-flow fecal coliform concentrations observed in these study streams, although other sources (including cross-pipes, failing septic sys

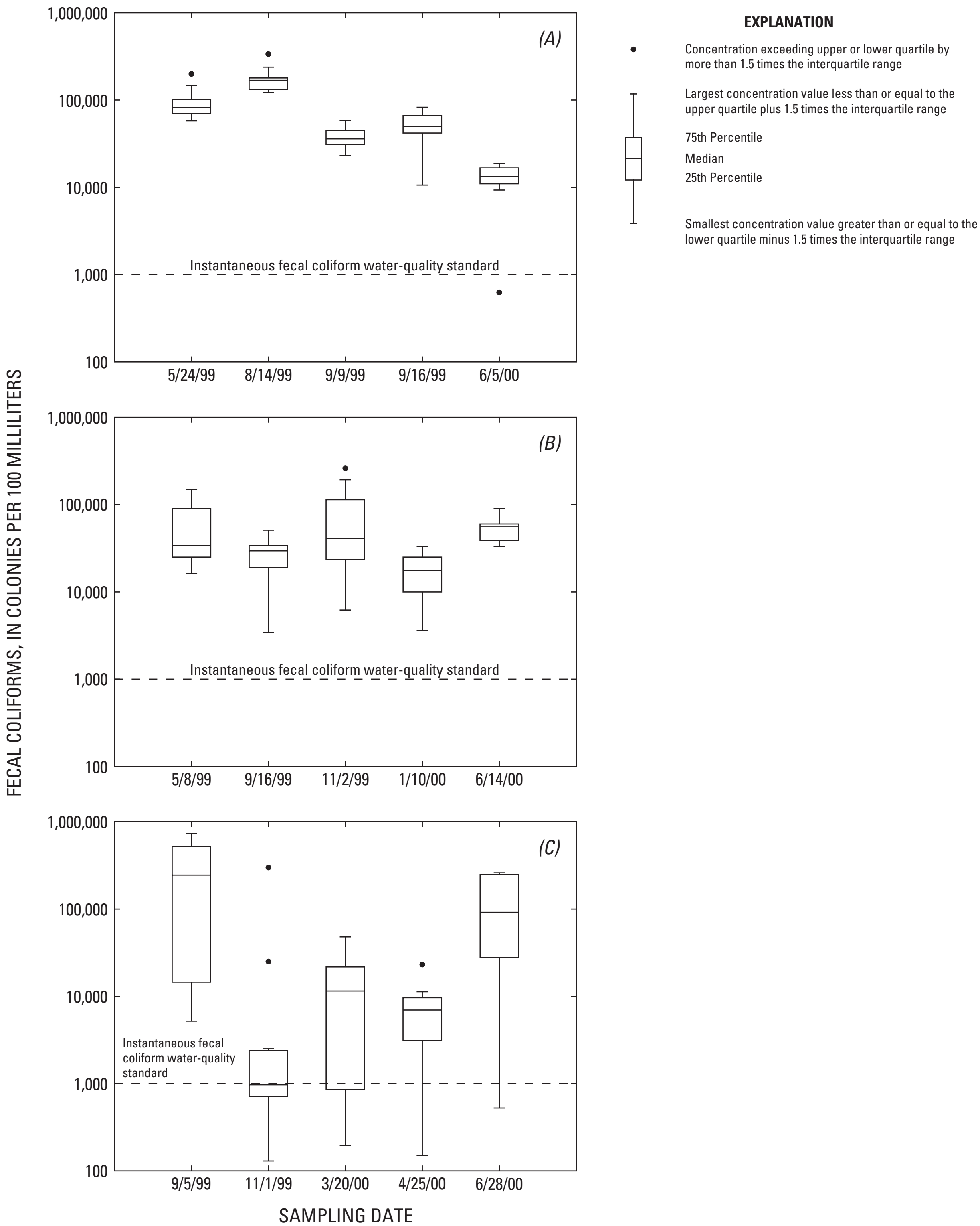


Figure 9. Fecal colifrom concentrations during storm-flow sampling of Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)* watersheds, Virginia, 1999-2000.

tems, and leaking or overflowing sewer lines) also may have contributed during storms.

Overall, the storm-flow responses of the fecal coliform bacteria and the supporting water-quality parameters were consistent among the three study streams and with the responses observed in previous studies. The data from the intensive sampling of the storm events are presented as a series of chemographs (figures demonstrating the time-course evolution of the stream-water composition during a storm event); a single, representative storm event from each study stream is presented (fig. 10). In general, the data demonstrated an increased fecal coliform concentration on the rising limb of the storm hydrograph, peak fecal coliform concentrations around the hydrograph peak, and decreased fecal coliform concentrations on the falling limb of the hydrograph. In a minor variation of this pattern, fecal coliform concentrations in Accotink Creek usually were slightly decreased during the peak in the hydrograph. Data on the supporting water-quality parameters (turbidity, specific conductance, pH, and dissolved oxygen concentration) were typically collected during storm events at a frequency slightly greater than that used for fecal coliforms. Turbidity levels always increased during storm events, generally reaching a maximum concentration about the time of the peak discharge. Increased turbidity levels are reflective of the suspended sediments that enter the water column because of surface runoff, re-suspension of the streambed sediments, or stream bank erosion (Kronvang and others, 1997; Jeje and others, 1991). The pH generally decreased slightly during storm events in all streams. Declines in pH are commonly observed during storms, as relatively more acidic rainfall, runoff, and interflow contributes to the streamflow. These acidic contributions consume buffering capacity and reduce the overall pH (Whitfield and others, 1993; Gburek and Pionke, 1993). As observed in earlier studies (Laudon and Slaymaker, 1997; Caissie and others, 1996), specific conductance generally decreased during storm events–an indication that the new water that was added to the stream during the storm had a relatively lower specific conductance than what was already resident in the stream. Although the initial runoff (also referred to as the “first flush”) from a watershed may contain relatively high concentrations of dissolved material (and an elevated specific conductance), subsequent runoff and incident rainfall generally are much more dilute and result in an overall reduction in the streamwater specific conductance during storm events (De Boer and Campbell, 1990). Dissolved oxygen concentrations typically decreased during storm events, a response that has been observed in other studies (Bolstad and Swank, 1997). This decrease in dissolved oxygen concentrations generally is attributed to rapid inputs of readily degraded organic material in the surface runoff, and potentially an increased oxygen demand by the re-suspended streambed sediments.

Correlations between fecal coliform concentrations and stream-water parameters

Correlations were examined between the observed fecal coliform concentrations and the supporting streamwater parameters to develop multiple linear regression models for predicting fecal coliform concentrations at each of the ambient water-quality sampling stations. Parameters considered for these empirical models included discharge, specific conductance, turbidity, pH, water temperature, and dissolved oxygen concentration. The multiple linear regression models were developed using the approach described by Helsel and Hirsch (1992). On the basis of their sample distributions, the fecal coliform concentration, discharge, and turbidity variables were transformed logarithmically (log base 10) to reduce skew and produce more normally distributed residual and partial plots. Best subsets regression was used to identify the most promising multiple linear regression models. These candidate models were subsequently screened for significance of all variables, and the best models were selected based on a minimized Mallows Cp and maximized adjusted R^2. Plots of the model residuals also were evaluated to ensure that the residuals were normally distributed and had a constant variance.

Although best subsets regression is the optimal method for developing multiple linear regression models, stepwise multiple linear regression also may be used (Helsel and Hirsch, 1992). As confirmation, stepwise multiple linear regressions also were performed on the fecal coliform concentration data, and the same supporting streamwater parameters were used as independent variables. The stepwise multiple linear regressions identified the same models as those selected using the best subsets regression.

Multiple linear regression models were developed for the ambient water-quality sampling station of each individual study stream, as well as a combined overall model of all three monitoring stations. These models predicted fecal coliform concentrations as a function of

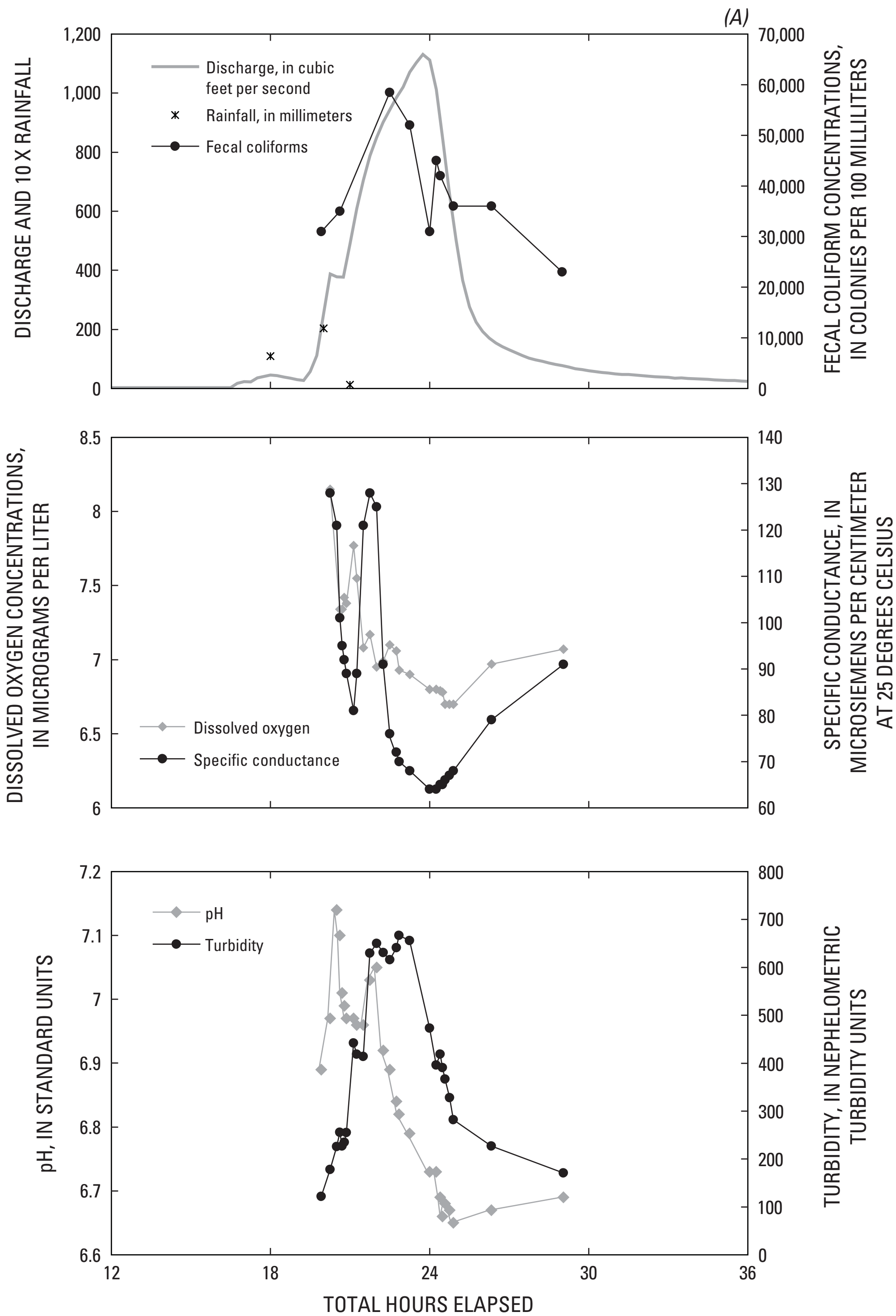


Figure 10. Changes in discharge, fecal coliform concentrations, and supporting water-quality parameters during storm events September 9-10, 1999, Accotink Creek *(A)*, September 15-16, 1999, Blacks Run *(B)*, and June 27-29, 2000, Christians Creek *(C)* watersheds, Virginia.

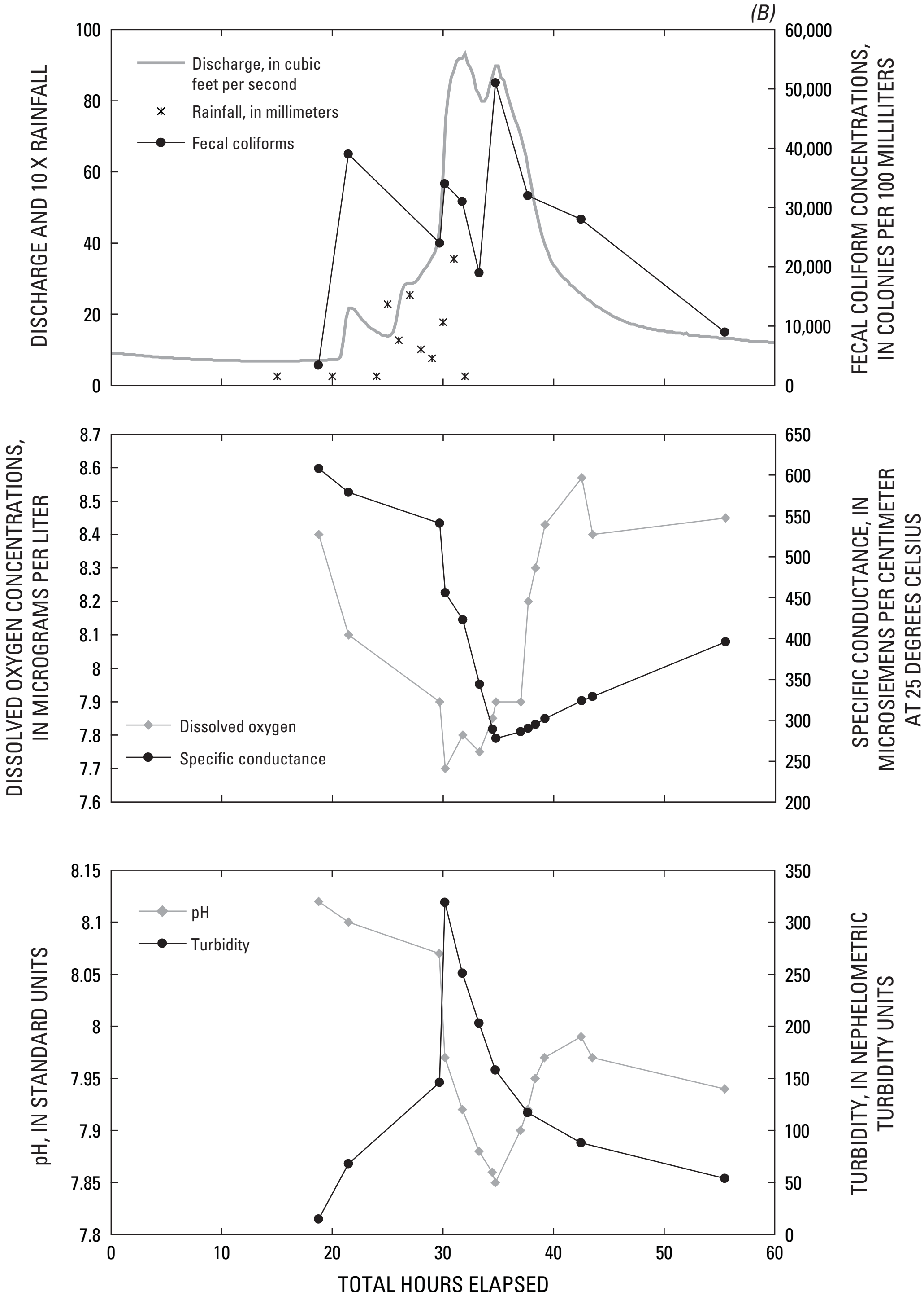


Figure 10. Changes in discharge, fecal coliform concentrations, and supporting water-quality parameters during storm events September 9-10, 1999, Accotink Creek *(A)*, September 15-16, 1999, Blacks Run *(B)*, and June 27-29, 2000, Christians Creek *(C)* watersheds, Virginia—Continued.

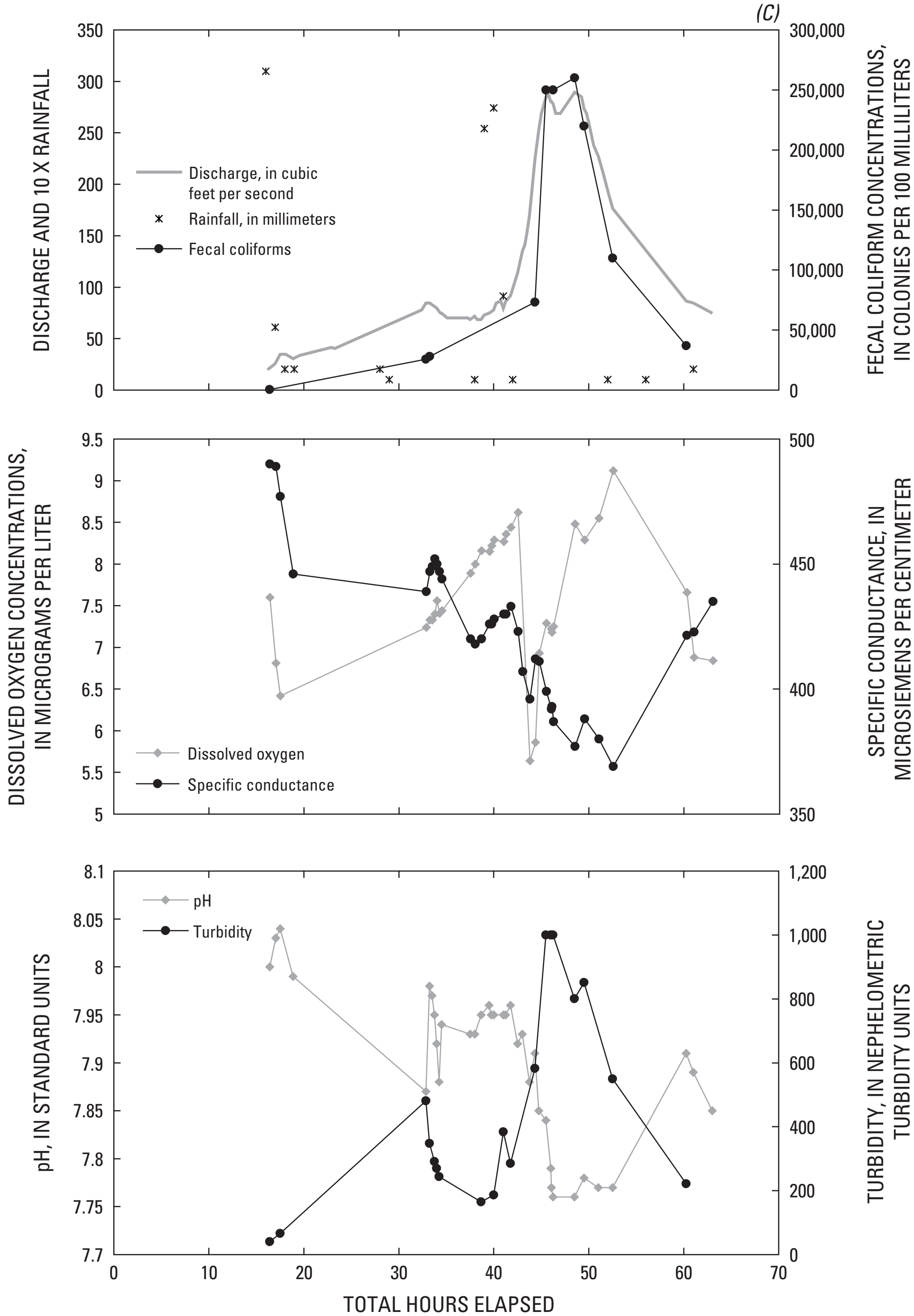


Figure 10. Changes in discharge, fecal coliform concentrations, and supporting water-quality parameters during storm events September 9-10, 1999, Accotink Creek *(A)*, September 15-16, 1999, Blacks Run *(B)*, and June 27-29, 2000, Christians Creek *(C)* watersheds, Virginia—Continued.

some of the supporting streamwater parameters. In all the models, turbidity was identified as the parameter that explained the greatest variance and was most significant in the model. In addition to turbidity, parameters such as water temperature, pH, and dissolved oxygen also were useful in explaining some of the variability in the fecal coliform concentrations. The regression equations and correlation coefficients for these models are:

Accotink Creek:

$$\log[FC] = 1.130\,(\log[Turb]) + 0.044\,(WT) + 1.068 \qquad (2)$$
$$R^2 = 0.88$$

Blacks Run:

$$\log[FC] = 0.768\,(\log[Turb]) - 0.086\,(DO) - 0.025\,(WT) + 3.825 \qquad (3)$$
$$R^2 = 0.68$$

Christians Creek:

$$\log[FC] = 1.314\,(\log[Turb]) + 0.668\,(pH) - 3.908 \qquad (4)$$
$$R^2 = 0.64$$

All three streams combined:

$$\log[FC] = 1.222\,(\log[Turb]) + 1.688 \qquad (5)$$
$$R^2 = 0.71,$$

where *FC* represents the fecal coliform concentration,
Turb is the turbidity,
WT is the water temperature, and
DO is the dissolved oxygen concentration.

A comparison between the model predictions and the observed data for all sites is presented (fig. 11). In general, these models explained between 64 percent and 88 percent of the observed variability in fecal coliform concentrations, depending on the study stream. The ability to predict fecal coliform concentrations from these easily measured water-quality parameters is useful, particularly when estimates of fecal coliform concentrations are needed quickly (18-22 hours of incubation are required before fecal coliform concentrations can be determined). Additionally, the parameters used in these predictive models are easier and less expensive to analyze for than fecal coliform; this regression approach may be especially useful in cases where monitoring cost is a special concern. Although it appears that these empirical models can be used to predict fecal coliform concentrations, independent verification is needed before these models should be applied. After verification, these models would be relevant to the conditions and streams in which they were developed. Given the variability in the observed fecal coliform concentrations (relative to the predicted concentrations), these empirical models may be best suited for situations that require only an approximate fecal coliform concentration, or that call for evaluating the likelihood of a water sample exceeding a specific water-quality standard or criterion.

Correlations between turbidity and fecal coliform concentrations have been observed previously (Christensen and others, 2001; Francy and Darner, 1998). Conceptually, the strong relation between fecal coliform concentrations and turbidity may result because both constituents are “flushed” into the stream during storm events (fecal coliforms are transported in runoff from parking lots, pastures, fields, and other surfaces; sediments are generally eroded off the land surface and carried into the stream). A distinction must be made, however, between this correlation and any inference of causality. Although turbidity is an effective predictor of fecal coliform concentrations, it cannot be inferred that the sediments (measured as turbidity) are the primary source of the fecal coliforms; rather, it can be concluded that conditions that favor elevated fecal coliform concentrations also favor elevated turbidity levels.

Analysis of replicate fecal coliform enumerations

Knowledge of the variability inherent in the fecal coliform enumeration process is important for comparing fecal coliform concentrations of different water samples. Replicate fecal coliform enumerations were performed on 6 percent of the samples collected (7 duplicate fecal coliform enumerations and 24 triplicate fecal coliform enumerations). The replicate fecal coliform enumerations were generally performed as multiple analyses of a single water sample (in a few cases, paired water samples were collected simultaneously and analyzed as duplicate samples). These replicate enumerations were analyzed using a percent difference calculation, given as:

$$\text{Sample \% difference} = \frac{(\text{Sample concentration}) - (\text{Mean of replicate})}{(\text{Mean of replicate})} \times 100\% \qquad (6)$$

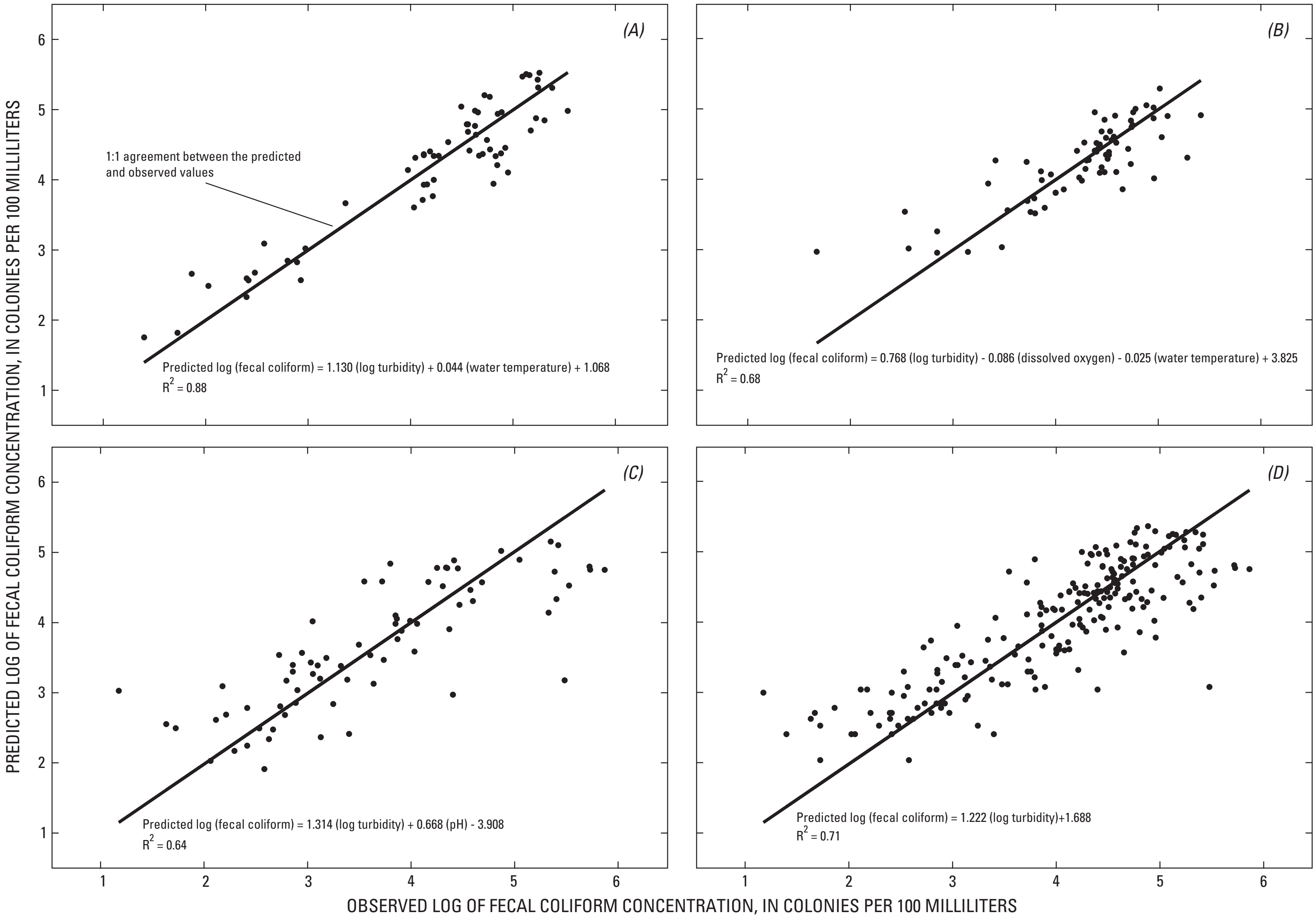


Figure 11. Relations of observed and predicted fecal coliform concentrations as a function of water-quality parameters, from streamwater samples collected March 1999 through October 2000 for Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, and all three streams combined *(D)*.

The percent difference values for each individual enumeration (n=86) were summarized to evaluate the variability in the fecal coliform enumeration technique. The percent difference term was normally distributed, with a mean percent difference value of 0.00 percent and a standard deviation of 12.2 percent. This normal distribution of the percent difference term can be used to calculate the probability of observing the specific percent difference value that is present between two fecal coliform concentrations (Johnson and Bhattacharyya, 1985).

Bacteria sources in the three streams

Samples submitted for source tracking

In performing this BST study, a large number of samples were collected over 20 months. Only the water samples collected from the ambient water-quality sampling station of each study stream were submitted for the source-tracking analysis; none of the continuum samples were submitted for ribotyping. This source tracking design was selected because it allowed the development of an understanding of the spatial and temporal patterns in fecal coliform concentrations throughout each study stream and it provided knowledge of the bacteria sources affecting water quality at the ambient water-quality sampling station for each stream.

Results of the bacterial source tracking

A total of 1,285 unknown *E. coli* isolates was ribotyped from the three watersheds during this investigation (table 5). Overall, 65 percent of those isolates were matched to a known-source isolate in the source library. Identification of 65 percent of the unknown isolates is considered successful and is consistent with previous ribotyping studies (Farag and others, 2001; Samadpour and Chechowitz, 1995). The distribution of the number and the type of isolates that were ribotyped is presented in table 5. About 61 percent of the source-tracked isolates were selected from low-flow samples, and about 39 percent of all isolates were from storm-flow samples. Similarly, about 59 percent of the identified *E. coli* were from low-flow samples, and 41 percent were from storm-flow samples. The collection and identification of *E. coli* isolates from both low-flow and storm-flow periods were important for identifying the dominant sources of bacteria in the watersheds.

Procedures for quantifying and interpreting BST data are still being developed; few standard protocols exist to handle the complexities of these data and the methods used to generate them (Simpson and others, 2002). As this technology is applied under different field settings and as the science of BST matures, more uniform approaches may be developed. One unresolved issue involves the number of known-source isolates that are needed to accurately quantify the distribution of bacteria sources. A sample size of about 1,000 *E. coli* isolates represents only a small fraction of the total number of fecal coliform bacteria that are transported by the three streams. The frequency with which samples should be collected during any BST study is also unresolved. More frequent sampling is expensive but may be necessary for evaluating seasonal patterns that may be present in the bacteria sources that are contributing to a stream. The value of storm-flow sampling is unresolved. Point sources are likely to be the primary contributors of fecal coliform bacteria to a stream during base-flow conditions, and nonpoint-source contributions likely dominate during storm-flow periods, but these patterns have yet to be investigated. There remain questions regarding the number of bacteria isolates to source-track from each individual water sample. Evaluating many isolates from a single water sample

Table 5. Number of *E. coli* isolates ribotyped, and percentage of those isolates from low-flow samples collected from three watersheds in Virginia, March 1999 through October 2000. Number (and percentage) of isolates that were identified, and the number (and percentage) of identified isolates from low-flow and high-flow samples

Watershed	Total isolates (percent low flow)	Identified isolates (percent)	Identified low-flow isolates (percent)	Identified storm-flow isolates (percent)
Accotink Creek	404 (64.6)	279 (69.1)	174 (62.4)	105 (37.6)
Blacks Run	451 (60.1)	285 (63.2)	173 (60.7)	112 (39.3)
Christians Creek	430 (59.5)	274 (63.7)	146 (53.3)	128 (46.7)
Total	**1,285(61.3)**	**838 (65.2)**	**493 (58.8)**	**345 (41.2)**

may provide a more detailed understanding of that particular sample, but restrictions in the scope of a study may result in fewer water samples collected and source-tracked. Although these questions remain unresolved, our intensive sampling over a 20-month period, incorporation of low-flow and storm-flow sampling, and identification of more than 270 isolates in each watershed should allow these data to be treated in a semi-quantitative manner and for inferences to be drawn regarding the bacteria sources that are impairing these three streams.

Before presenting the bacteria sources that were identified in the three watersheds, the unidentified *E. coli* isolates must be considered. Approximately 35 percent of the isolates were unidentified. These unidentified isolates represent *E. coli* that were not yet present in the known-source library. Based on knowledge of the potential fecal coliform contributors in these watersheds and the sources represented in the known-source library, the presence of a significant yet unrepresented fecal coliform contributor in these watersheds (lions, for example) is unlikely. It is likely that the unidentified isolates are from sources that are common in these watersheds (humans, dogs, and raccoons, for example) but that the particular ribotype was not yet included in the known-source library. Collection of additional known-source isolates likely would reduce the number of unidentified isolates. On the basis of the diversity of the 50,000-isolate known-source library that was used in this study, it is reasonable to assume that the sources of the unidentified isolates had a distribution that was identical to the source distribution observed in each watershed. The implication of this assumption is that the identified isolates could be used to describe the overall distribution of *E. coli* sources (and, therefore, fecal coliform sources) that impaired each watershed.

The identified bacteria sources in the three watersheds demonstrate that a diverse collection of fecal sources contributed to the impairment of each stream (fig. 12). Two source categories are discussed in more detail. The first source category that was treated differently is poultry, which represents a combination of chicken and turkey sources. The ribotyping technique sometimes was able to distinguish chickens from turkeys (and the two are labeled separately in figure 12b and c); in other cases, an isolate was identified as either a chicken or a turkey isolate (in this case, the isolate is labeled as poultry). This lack of specificity may have occurred for three reasons: (1) identical *E. coli* were found in both birds; (2) different *E. coli* were found in chickens and turkeys, but the ribotyping analysis produced banding patterns that were identical; or (3) the ribotype from the source library that matched the unknown isolate was identified during the source collection process as poultry litter and did not indicate whether the sample was from chickens or turkeys. For data-interpretation and watershed-modeling purposes, the chicken, turkey, and poultry categories were combined into a total poultry category. The second category that was treated differently is avian, a source which was identified in all three watersheds. The avian category represents *E. coli* isolates that occurred in multiple bird species. Whereas the poultry category is specific to chickens and turkeys, the avian category encompasses all birds. For data-interpretation and watershed-modeling purposes, this avian category was distributed among all the observed bird sources, which included geese, ducks, sea gulls, crows, poultry, and swans. Quantitatively, it was assumed that the avian component was distributed proportionally, according to the occurrence of each individual bird source shown in figure 12. For example, if the goose contribution for an individual stream was 25 percent of all the bird sources that were identified, then 25 percent of the avian contribution was attributed to geese. In this way, the avian contribution was distributed among all the identified bird sources.

After combining the poultry sources and distributing the avian component, the *E. coli* sources of each stream were re-plotted (fig. 13). The plot for each stream was arranged from the greatest contributor to the least contributor. No single source accounted for more than 30 percent of the identified *E. coli*; a range of sources contributed fecal coliforms to all three stream systems. In Accotink Creek, the greatest contributors were geese and human sources, followed by dogs, ducks, cats, sea gulls, and raccoons (fig. 13a). Cattle, poultry, human sources, and dogs were the top four sources in both Blacks Run and Christians Creek (fig. 13b and c). Cats also were an important source in Blacks Run, whereas horses and deer were additional sources to Christians Creek. All other observed sources were minor, providing less than 5 percent of the total source observed in these streams. Although they were independently considered minor, these minor sources may be cumulatively important to the overall water quality in these streams.

The bacteria-source data can also be grouped by their general animal categories (humans, pets, waterfowl, wildlife, and agricultural; fig. 14). Accotink

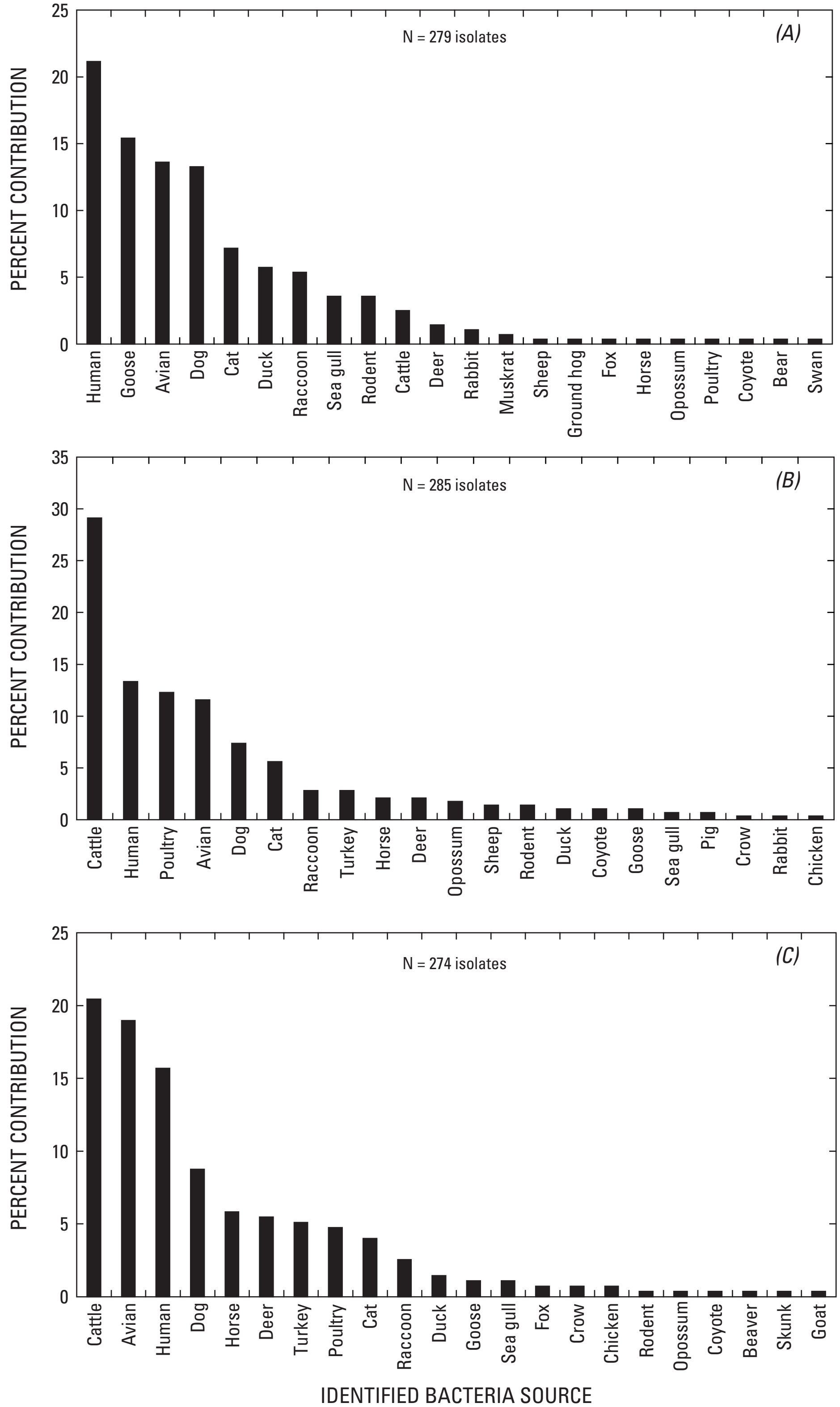


Figure 12. Distribution of the bacteria isolates that were identified in streamwater samples collected from March 1999 through October 2000 in Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, Virginia.

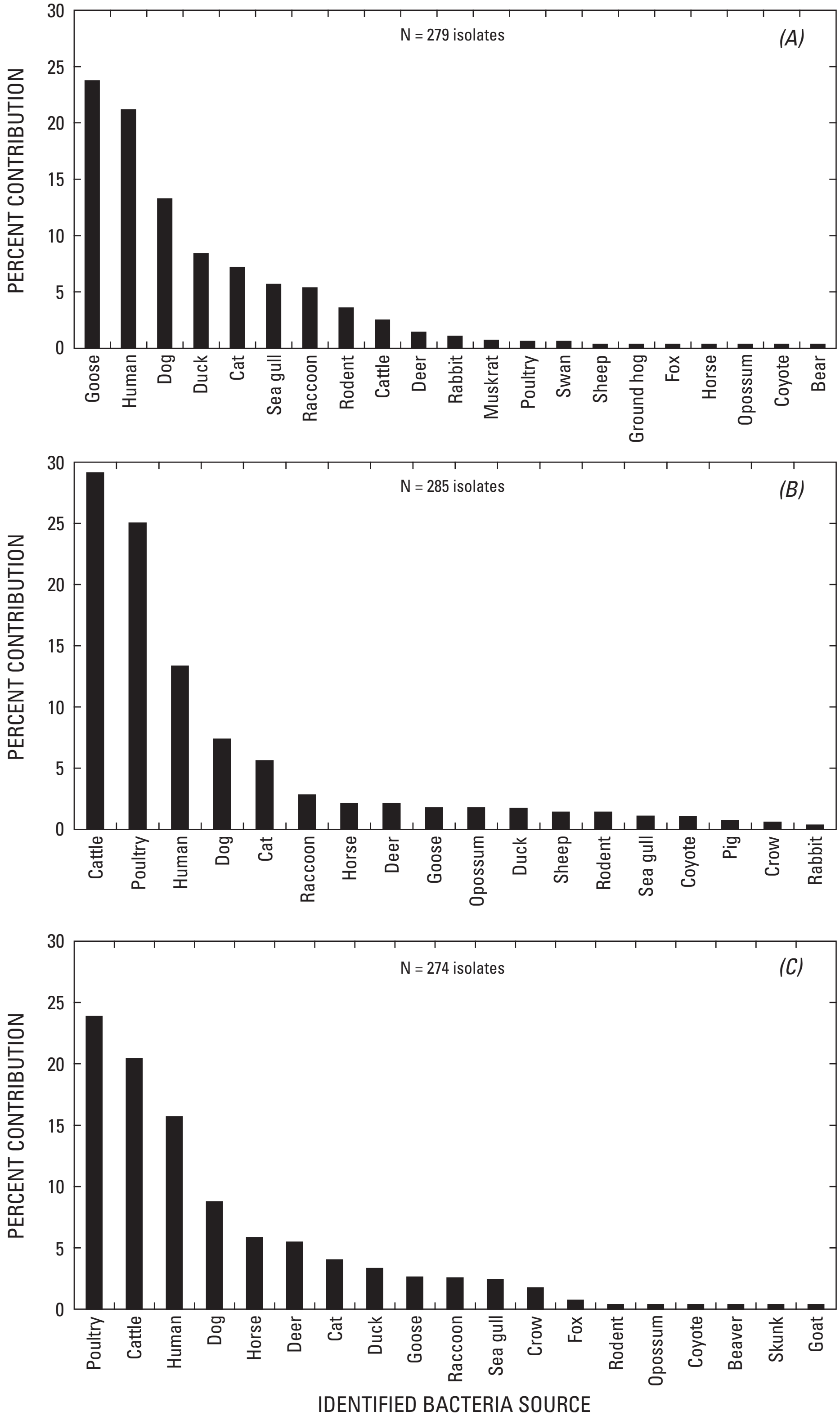


Figure 13. Distribution of the bacteria isolates that were identified in streamwater samples collected from March 1999 through October 2000 in Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, Virginia, after combining the poultry sources and distributing the avian source.

Creek was dominated by waterfowl sources (geese, ducks, sea gulls, and swans), followed by almost equal contributions from human sources and pets (dogs and cats). Wildlife also made an important contribution to Accotink Creek, whereas agricultural sources were relatively minor. Both Blacks Run and Christians Creek were dominated by agricultural sources, followed by contributions from human sources, pets, and wildlife. Both Blacks Run and Christians Creek also had relatively minor contributions from waterfowl. In addition to the differences in the general categories that contributed to the impairment of each stream, the data indicate that a range of sources contributed fecal coliforms to each stream; no one group of sources accounted for more than 60 percent of the identified *E. coli* in these stream systems.

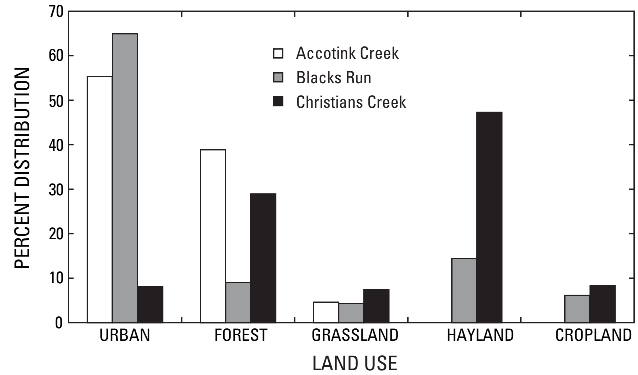


Figure 15. Land use in the Accotink Creek, Blacks Run, and Christians Creek watersheds, Virginia. Urban represents residential and commercial uses; grassland in the Accotink Creek watershed is primarily parks, golf courses, and residential lawns; grassland in the Blacks Run and Christians Creek watersheds is primarily pastureland. See figures 2-4 for sources of land-use data.

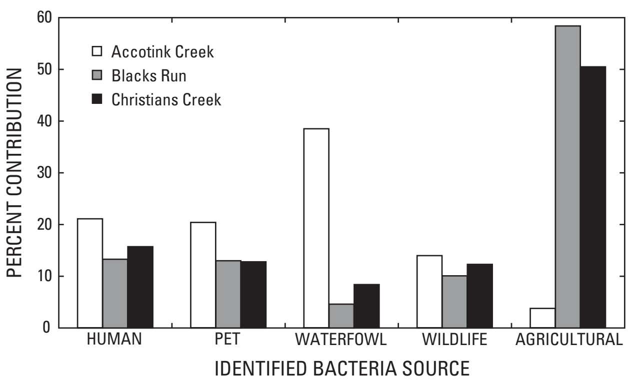


Figure 14. Bacteria sources identified in streamwater samples collected March 1999 through October 2000 from three watersheds in Virginia, grouped by animal category.

Comparison of the BST results (figs. 13 and 14) with the land use of each watershed (fig. 15) demonstrates relations between the dominant activities within each watershed and the observed bacteria sources. The land use of each watershed can be used to infer the source category that would be expected to contribute bacteria to these three streams. Although information about the land use can aid in verifying the presence of an observed source, the BST data from this study do not provide information on the specific mechanisms by which the bacteria are entering these streams.

The Accotink Creek watershed is primarily urban, but still contains large amounts of forested areas and smaller amounts of open, grassland areas; its bacteria sources reflect this land-use pattern. The human population in the watershed is estimated to be about 110,000; therefore, the presence of human-source bacteria is not surprising. It is unknown, however, whether this human waste source is contributed by failing septic systems, leaking sewer lines, cross-connected sewer and storm drains, or straight pipes. Similarly, the dominant contributions from waterfowl are not surprising, given the large resident goose and waterfowl populations in the watershed. Waterfowl populations in the area are large because of an abundance of golf course ponds, development lakes, public parks, and other standing water bodies throughout the watershed. The proximity of waterfowl to the stream (and its tributaries) is also likely an important component of the large waterfowl contribution. The significant contributions from dogs and cats are indicative of a large pet population. Wildlife was also an important contributor, and wildlife populations have adapted to both the urban and forested areas of this watershed.

Land use in the Blacks Run watershed reflects the urban activities of the city of Harrisonburg and the agricultural activities that dominate the downstream portions of the watershed. The human population of the watershed is approximately 34,700, providing a source of human waste that could enter the stream through multiple pathways. Agricultural activities in the watershed are demonstrated by the areas of cropland, hayland, and pastureland; however, the agricultural activities also include intensive cattle and poultry farming (County of Rockingham, Department of Planning and Zoning, 1997). The intensive cattle and poultry farming are likely the source of the cattle and poultry contributions in Blacks Run; however, the mechanisms by which these bacteria are transported into the stream are uncertain. Erosion of field-applied manure is one potential mechanism. Direct deposition of waste into the stream by cattle (in areas where cattle have direct access to the stream) also may be important. Human activities in both the urban and agricultural areas are likely responsible for the pet contributions of bacteria to the stream.

The Christians Creek watershed is dominated by agricultural activities and forested areas; urban areas are minimal. The human population of the watershed is approximately 12,000—considerably smaller than either of the other two watersheds. Although fewer people live in the Christians Creek watershed, *E. coli* of human origin were detected and were an important contributor to the stream. Christians Creek may have a higher occurrence of near-stream contributors than the other study streams. Three straight pipes have been identified in the watershed; these pipes may route untreated wastewater from three houses directly into the stream. These three straight pipes may or may not be contributing an appreciable quantity of the human *E. coli* that are observed at the ambient water-quality sampling station; however, they demonstrate the potential for other straight pipes and a condition in which a single, near-stream source (a straight pipe, for example) may contribute more bacteria than another mechanism (numerous failing septic systems that are located a considerable distance away from the stream, for example). Human activities in the watershed are likely responsible for the pet contributions of *E. coli* to the stream. Agricultural practices are dominant in this watershed; however, the density of these agricultural activities is lower than in the Blacks Run watershed. Similar to Blacks Run, cattle and poultry production accounts for the primary livestock populations in the watershed; these livestock generate large amounts of feces that may be routed into the stream. Numerous horse farms are also located in this watershed, providing a source for the horse waste in the stream. The mixture of forested and agricultural land produces a habitat that is conducive to populations of white-tailed deer and other wildlife.

As an emerging technology, published BST studies are limited; however, other studies have presented field results that can be compared to the results from this study. Four Mile Run (a nearby watershed, approximately 5 miles east of Accotink Creek) was studied by Simmons and others (2000). The Four Mile Run watershed has similar land-use practices and watershed characteristics as Accotink Creek. Simmons and others (2000) used a different method of BST (pulsed-field gel electrophoresis) and a different sampling protocol than used here; however, their identified bacterial sources were similar to those observed in Accotink Creek (fig. 16). Waterfowl, human sources, and dogs all were identified as major contributors of bacteria to both systems. Even though less similar contributions were observed for raccoons, deer, and cats, both studies identified these animals as contributors. Studies in analogous watersheds are not available for direct comparison with the study results in Christians Creek (the agricultural watershed) and Blacks Run (the mixed urban and agricultural watershed); however, others have performed source-tracking (using antibiotic resistance analysis) studies in agricultural watersheds. Cattle have been identified as the primary contributor of fecal coliform bacteria in some agricultural watersheds in Virginia (Hagedorn and others, 1999; Wiggins, 1996). Although the contributions were less than those observed in Christians Creek and Blacks Run, Wiggins (1996) also documented bacteria contributions from both poultry and human sources in some agricultural watersheds.

Despite the wide-spread occurrence of elevated fecal coliform concentrations in surface waters, this water-quality condition appears to be reversible. In two watersheds (one dominated by wildlife sources, the other dominated by agricultural sources), previous studies demonstrated that reducing the dominant sources of fecal pollution identified by BST methods may result in significantly improved water quality (Hagedorn and others, 1999; Simmons and others, 1995). Hagedorn and others (1999) observed an average fecal coliform concentration reduction of 94 percent following the implementation of source-control measures.

Temporal variability in the bacteria sources

The effects of flow on the distribution of bacteria sources were evaluated by comparing the distribution of bacteria sources during low-flow periods and storm-flow periods (fig. 17). It was expected that the bacteria sources would differ between these low-flow and high-flow periods as runoff processes occurred and waste from different sources was flushed into the streams. Although there were small variations in the source contributions, the data indicated that distributions of bacteria sources were relatively uniform during both sampling periods; major contributors during low-flow periods were major contributors during

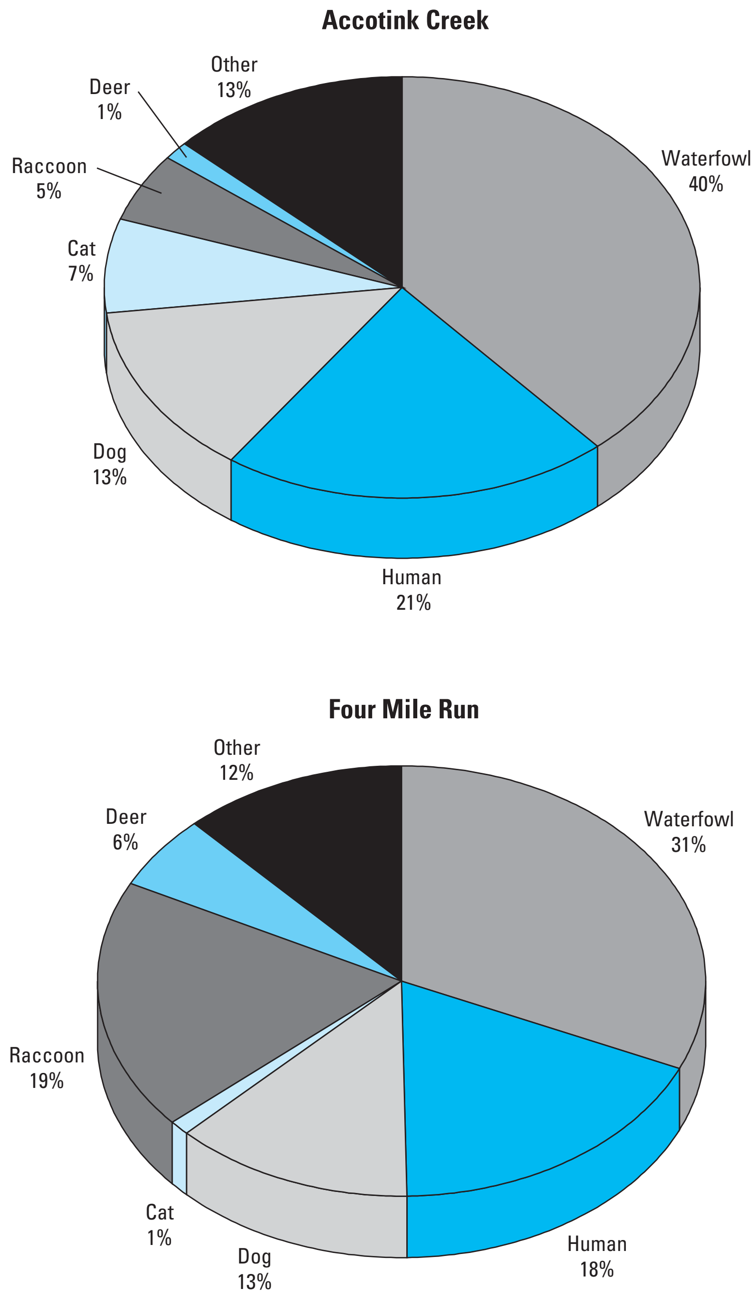


Figure 16. Distribution of identified bacteria sources in two neighboring watersheds, Accotink Creek and Four Mile Run, Virginia. (Four Mile Run data from Don Waye, Northern Virginia Regional Commission, written commun, 2001.)

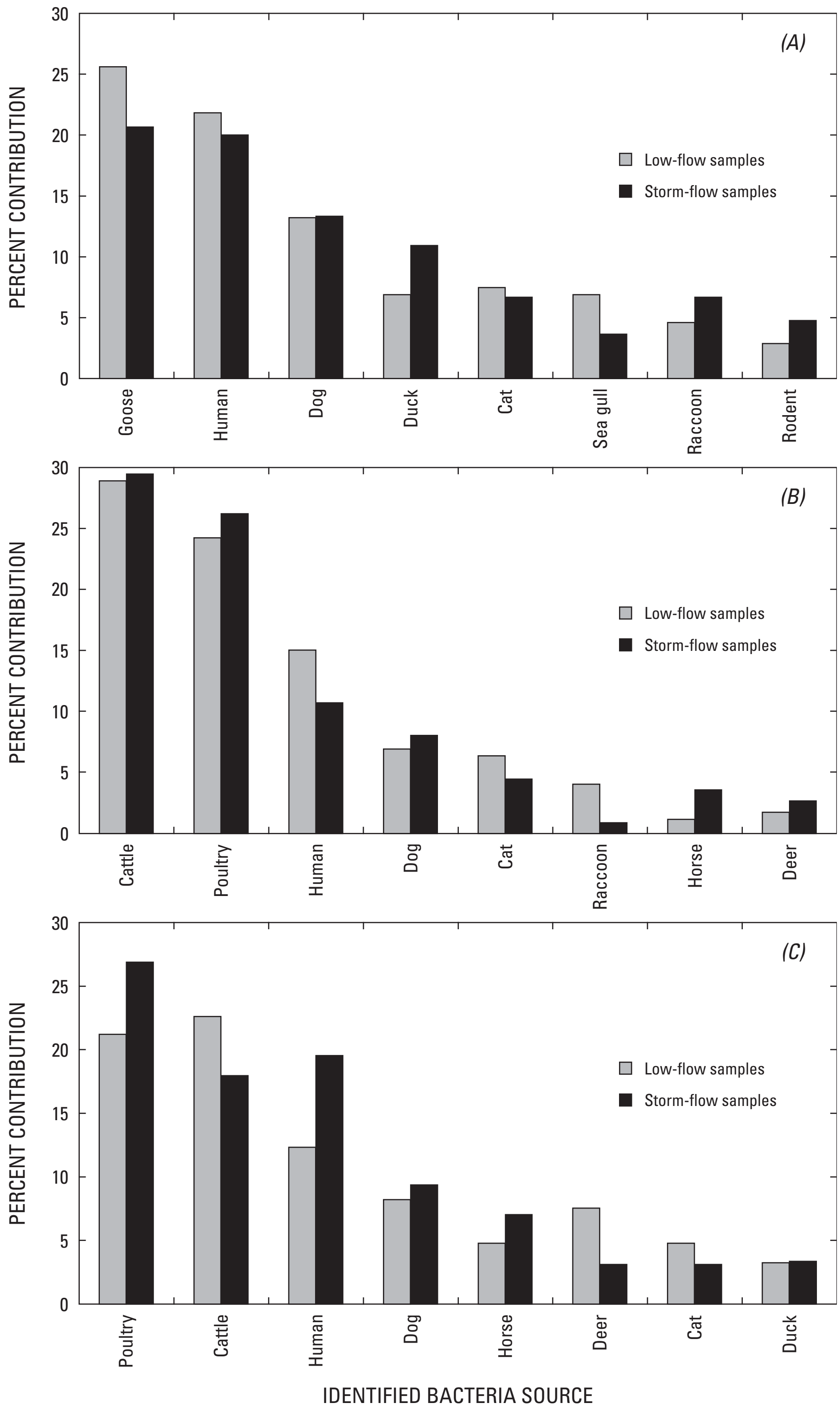


Figure 17. Top eight bacteria sources from low-flow and storm-flow streamwater samples collected March 1999 through October 2000 in Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, Virginia.

storm-flow periods, and minor contributors during low-flow periods were minor contributors during storm-flow periods. Although no statistical analysis was performed to establish error bars on these plots (fig. 17), the relatively small number of isolates in each flow category and data analysis indicate that differences of 5 percent or less would be inconclusive. Using this criterion, the Christians Creek data indicated that there might be a slight increase in poultry and human sources during storm events; however, these increases were not indicated in either the Accotink Creek or Blacks Run data.

The observation of relatively uniform distributions of bacteria sources during both low-flow and high-flow periods remains largely unexplained. This pattern may indicate that the bed-sediment reservoir of these streams is a significant source of fecal coliform contamination in the water column. In this scenario, a "sloughing off" of bacteria from the bed-sediment surface may produce the low-flow distributions of fecal coliforms. During storm-flow periods, these same bed sediments are re-suspended into the water column. If no other factors were affecting the streamwater fecal coliform bacteria composition, this situation would result in similar distributions of low-flow and storm-flow bacteria sources. Because streamflow generation, suspended-sediment transport, and fecal coliform transport are complex processes, however, this scenario is probably oversimplified. Alternatively, the complex runoff processes that are initiated during storm events may combine to produce a similar bacteria source distribution to that observed in these three streams during low-flow periods. To our knowledge, no other studies have reported the effects of flow on bacteria-source distributions. The potential for a variation in the distributions of fecal coliform bacteria sources between low-flow and storm-flow periods requires further investigation.

Seasonal patterns in the bacteria-source distributions also were investigated (fig. 18). To have enough isolates in each seasonal category for a meaningful analysis, the seasonal evaluation only involved a comparison of the relatively warm months (April-September) with the relatively cool months (October-March). Only the low-flow samples were used for this analysis to ensure that slight differences between low-flow and storm-flow distributions were not misinterpreted as seasonal patterns. Although some variability was evident in the data, the Accotink Creek results failed to demonstrate seasonality. Seasonal patterns were not necessarily expected in Accotink Creek because the populations of fecal coliform sources in the watershed remain stable over the entire year. The Blacks Run data indicated seasonality in the poultry contributions, with higher percent contributions during the cool months and lower percent contributions during the warm months. This seasonal pattern is logical because the early spring and the late fall (the cool months) are generally when poultry litter is applied to the agricultural fields for fertilizer and as a method of waste disposal. If this field-applied manure were being washed off the fields and into Blacks Run, a larger poultry contribution would be expected during and immediately after application to fields. A similar seasonal pattern was also observed in Christians Creek; in addition to the increased importance of poultry contributions during cool months, however, there also appeared to be an increase in the percentage of cattle contributions during warm months. This seasonal pattern is consistent with the animal-management practices in the Christians Creek watershed. Similar to the Blacks Run watershed, poultry litter applications generally occur in the late fall and early spring. Many cattle herds had direct access to Christians Creek, and during the warmer months, cattle were observed wading into streams and spending many hours wallowing (and sometimes defecating) in the stream. During the cooler months, cattle still visited the streams as a water source, but their time spent in direct contact with the water was reduced greatly compared to the warmer months. This pattern of animal behavior would produce the observed relative dominance by cattle sources during the warm months and a shift to dominance by poultry sources during the cool months. A review of the Blacks Run data indicated a 7-percent increase in the cattle contributions during the warm months. Although this increase in the Blacks Run cattle contribution may not be significant, it lends additional support to the observed seasonal pattern. A similar increase in the contributions of cattle sources during the hot summer months was also observed by Bower (2001). Although the observed seasonal patterns in this study are consistent with the land-use and agricultural practices in each watershed, additional sampling and more detailed discretization (consideration of four seasons) would be needed to confirm these seasonal patterns and further explore the more subtle changes that might be occurring in the contributions from the less dominant fecal coliform sources.

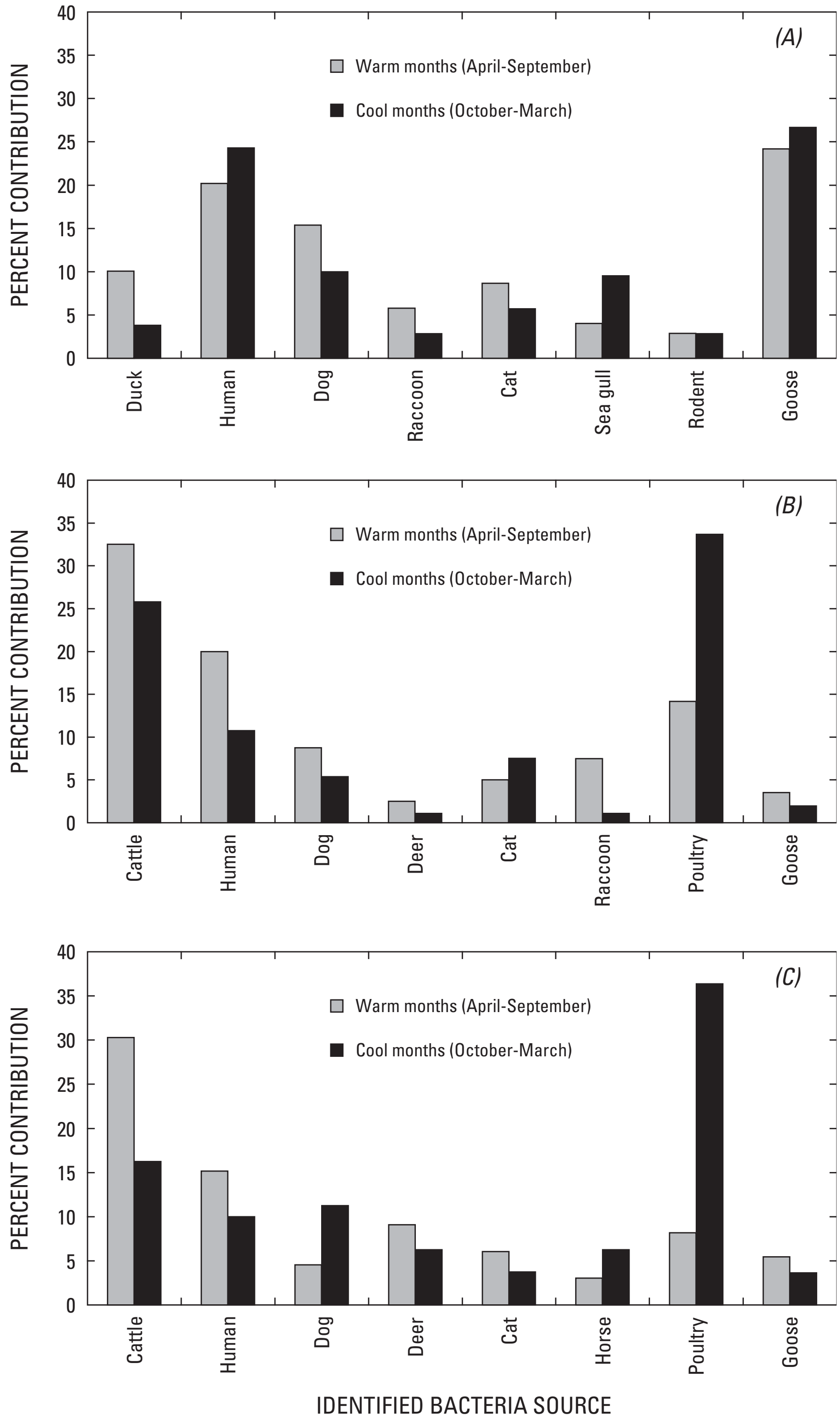


Figure 18. Top eight bacteria sources from low-flow streamwater samples collected April through September 1999 and October 1999 through March 2000 from Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, Virginia.

Quality control for the ribotyping results

Quality control for the ribotyping method was done through a blind isolate experiment. In this experiment, 23 known *E. coli* source isolates were randomly selected from the source library at the UWMSTL and sent to the USGS Virginia District for preparation and blinding. The 23 original source isolates were prepared as single, duplicate, or triplicate blind isolates and re-labeled with a key that was known only to USGS personnel (the number of blind isolates prepared from each original source isolate also was not revealed to the UWMSTL). A total of 66 blind isolates was then returned to the UWMSTL for ribotyping analysis. The UWMSTL used the ribotype patterns to identify which blind isolates were replicates (*E. coli* from the same original source isolate) and to match the blind isolate with the original source isolate from the known-source library (table 6). The UWMSTL successfully identified all replicate isolates and associated the blind isolates with the original 23 isolates from the known-source library. This quality-control experiment supports the capacity of the ribotyping method to generate reproducible, isolate-specific banding patterns, and supports the utility of ribotyping for fingerprinting *E. coli*.

The observations of poultry waste in Christians Creek and Blacks Run were supported by Hancock and others (2000), who examined arsenic concentrations in Christians Creek streamwater during both low-flow and storm-flow periods. The bedrock and soils of the Christians Creek watershed are not considered an arsenic source; however, feed amendments containing arsenic (such as Roxarsone, 3-nitro-4-hydroxyphenylarsonic acid) are commonly used in the poultry industry. The arsenic generally passes through the birds (Aschbacher and Feil, 1991) and is excreted with their feces (Morrison, 1969; Kunkle and others, 1981). Field application of poultry litter (which may contain this excreted arsenic) and transport during subsequent storm events may flush poultry-derived arsenic into the streams. Hancock and others (2000) found that detectable concentrations of total arsenic were present during low-flow conditions and that the total arsenic concentration increased during a storm event, supporting the hypothesis that field-applied poultry waste was flushed into streams. The poultry litter that was flushed into streams was also a likely source of the poultry contributions observed here.

In these streams, the presence of fecal coliform bacteria from humans was not unexpected; however, the identification of humans as one of the top three contributors in each stream was unexpected. The presence of human waste in these streams also was indicated by the presence of caffeine and cotinine, both of which can be used as chemical tracers of human wastewater (S.D. Zaugg, U.S. Geological Survey, written commun., 2002). Caffeine is a stimulant that is commonly found in many beverages (like coffee and soda) whereas cotinine is a metabolite of nicotine (the primary source being cigarettes). Some caffeine passes unchanged through the human body, whereas cotinine is produced as a metabolite; both compounds can then be excreted in human waste. Identification of these two compounds in streamwater is an indication of the presence of human waste, but does not indicate the mechanism by which the waste is entering the stream. During a single sampling of all three streams, detectable concentrations of both caffeine and cotinine were measured at the ambient water-quality sampling station of each watershed (fig. 19). Cotinine concentrations are estimated because of the method reporting limit. These data cannot be used to quantify the amount of human waste in

Table 6. Design of the quality-control experiment for the ribotyping analysis used in this study

[UWMSTL, University of Washington Microbial Source Tracking Laboratory; USGS, U.S. Geological Survey; –, no replicate]

UWMSTL library identification number	USGS replicate identification number		
24221	72	–	–
24269	70	2	–
25145	67	5	69
26102	64	8	66
26623	61	11	63
26830	58	14	60
13043	52	20	54
13083	49	23	51
13949	46	26	48
14229	43	29	45
14653	40	32	42
15894	37	35	39
16113	34	38	36
18762	28	44	30
18964	25	47	27
19446	22	50	24
19585	19	53	21
19966	16	56	18
22178	7	65	9
24183	1	71	3
17042	73	80	79
21075	84	81	86
24049	87	85	75

the streams, but they do provide additional, independent evidence of the presence of human waste in all three of these streams.

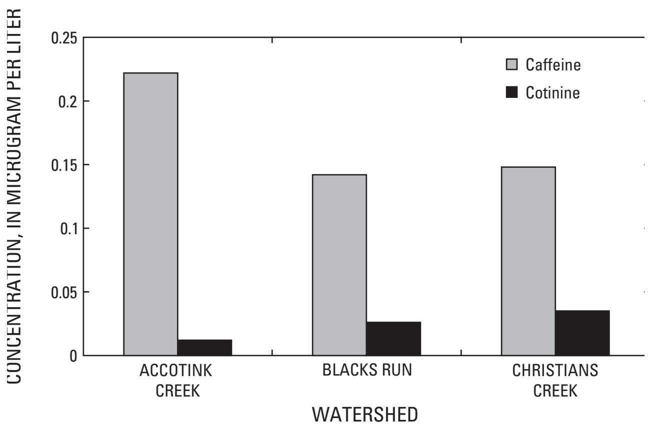


Figure 19. Caffeine and estimated cotinine concentrations measured in Accotink Creek on August 8, 2000, Blacks Run on August 15, 2000, and Christians Creek on August 1, 2000.

Source-library development and application

Successful application of *E. coli*-based BST methods requires the development of an extensive known-source library that represents all major contributors of feces to a particular watershed. The UWMSTL's ribotyping method involved direct comparison of known-source with unknown-source isolate banding patterns, with an exact match in the banding patterns required for positive source identification. Isolates that differed by even a single band were not considered matches. Because of these stringent matching requirements, this method cannot identify any isolates/ribotypes that are not already a part of the known-source database. Two known-source libraries were used in the study. These two libraries consisted of the UWMSTL's large database (containing approximately 50,000 isolates) and the UWMSTL's Virginia-specific database (containing approximately 450 isolates). The Virginia-specific library consisted of source isolates that were collected during previous investigations unrelated to this study.

To enhance the rate of positive source identification, 723 known-source samples were also collected from the three watersheds investigated in this study (table 7). Of these 723 samples, only 559 unique banding patterns were obtained (some of the isolates exhibited the same ribotype). These 559 unique isolates were then compared to the UWMSTL's large database and the Virginia-specific database. More than half (62.8 percent) of the site-specific source isolates that were collected during this study were already present in the UWMSTL's large database. Although the Virginia-specific database was relatively small (compared to the UWMSTL database), nearly 13 percent of the site-specific source isolates that were collected were already present in this database. Of the new known-source isolates collected, 4.3 percent were classified as transient strains of *E. coli* (strains that have been observed in more than one animal classification). Source samples from this study were compared with those already in the UWMSTL's large database and the Virginia-specific database; 27.5 percent of the isolates were identified as new ribotypes, added to the Virginia-specific source library, and used to identify the unknown isolates from this study. The large percentage of source isolates already present in the UWMSTL's large source database (62.8 percent) supports the conclusion that this database had national relevance and, therefore, a national database approach was reasonable for this ribotyping method. In addition, although many of the known-source isolates in this study were already included in the existing source libraries, the contribution of 154 new known-source isolates to the Virginia-specific source library was important and supports the need to collect site-specific fecal samples.

An examination of the databases used to identify the unknown isolates provided further support for using both a database of national scope and a site-specific database. For most cases, a record is available of which database was used to identify each unknown isolate (table 8). Most of the unknown isolates (60.5 percent) were identified using the UWMSTL's large database; however, an appreciable percentage of the unknowns (12.9 percent) were identified using only the Virginia-specific database (this database did include the 154 new known-source isolates that were collected as part of this study). A portion of the unknown isolates (16.1 percent) could be identified using either database, and in some cases (10.5 percent), the database used for the identification was inadvertently not recorded. These results highlight the utility of a large database for the ribotyping method; however, the results also demonstrate the need to supplement a large existing database with locally collected known-source isolates. If only one of these known-source databases had been used for identifying the unknown isolates, the number of identified isolates would have decreased considerably (from 65 percent to 29 percent if only the Virginia-specific

Table 7. Summary of source samples collected in the Accotink Creek, Blacks Run, and Christians Creek watersheds, Virginia, from March 1999 through October 2000, and comparison of the isolates from these source samples with the available source-library databases

[Unique source isolates identified represents the number of genetically distinct source isolates that were observed; this value is generally smaller than the number of source samples collected because clones were occasionally observed between source samples. The sum of the 5 columns to the right of the unique source isolates identified column is equal to this unique source isolates identified column; UWMSTL, University of Washington Microbial Source Tracking Laboratory]

Source	Number of source samples collected	Unique source isolates identified	Isolates already in the Virginia-specific database	Isolates already in the UWMSTL large available database	Isolates already in both databases	Isolates identified as transient	New source isolates added to the Virginia database
Human	220	168	4	103	15	7	39
Pets							
Dog	66	51	3	31	2	4	11
Cat	30	22	1	12	2	1	6
Livestock							
Cow	132	83	7	51	4	4	17
Turkey	39	39	3	22	2	0	12
Chicken	28	23	1	15	1	1	5
Horse	16	12	0	5	2	0	5
Sheep	5	5	0	2	0	2	1
Goat	3	3	0	0	0	1	2
Donkey	1	1	0	0	0	0	1
Mule	1	1	0	1	0	0	0
Pig	1	1	0	0	0	0	1
Poultry	1	1	0	0	0	0	1
Wildlife							
Goose	47	32	2	17	2	1	10
Duck	28	17	1	9	3	1	3
Deer	21	18	1	8	2	0	7
Muskrat	10	10	2	4	0	1	3
Groundhog	9	9	2	2	1	0	4
Rabbit	9	9	0	3	0	1	5
Squirrel	9	8	0	3	2	0	3
Fox	8	8	1	3	1	0	3
Opossum	7	6	0	3	1	0	2
Raccoon	5	5	2	1	1	0	1
Skunk	5	5	0	2	1	0	2
Hawk	4	4	0	3	0	0	1
Bird	3	3	0	2	0	0	1
Crow	3	3	0	2	0	0	1
Rat	3	3	0	1	0	0	2
Beaver	2	2	0	0	0	0	2
Pigeon	2	2	0	1	0	0	1
Osprey	1	1	0	1	0	0	0
Quail	1	1	0	0	0	0	1
Robin	1	1	0	0	0	0	1
Starling	1	1	0	1	0	0	0
Waterfowl	1	1	0	1	0	0	0
Totals	**723**	**559**	**30**	**309**	**42**	**24**	**154**
Percentages[a]		**100**	**5.4**	**55.3**	**7.5**	**4.3**	**27.5**

[a] Percentages are based on the number of unique source isolates identified.

Table 8. Summary of databases used to identify the source of each isolate for this study

[UWMSTL, University of Washington Microbial Source Tracking Laboratory]

Watershed	Total number of isolates	UWMSTL large database (percent)	Virginia-specific database (percent)	Both databases (percent)	Unspecified database (percent)
Accotink Creek	279	177 (63.4)	30 (10.8)	43 (15.4)	29 (10.4)
Blacks Run	285	176 (61.8)	43 (15.1)	43 (15.1)	23 (8.1)
Christians Creek	274	154 (56.2)	35 (12.8)	49 (17.9)	36 (13.1)
Total	**838**	**507 (60.5)**	**108 (12.9)**	**135 (16.1)**	**88 (10.5)**

database had been used for the source identification). The large size of the UWMSTL database is likely the reason it was able to identify the majority of the unknown isolates; the percentage of isolates identified likely would have increased if an even larger known-source database had been used. Although the size of the UWMSTL large database is important, the local nature of the Virginia-specific database is also important. In general terms, the fecal sources that have been sampled for the Virginia-specific source library should be more similar to the actual fecal sources that are found in Virginia waterways. Based on this work, the best source tracking results are likely produced from a coupled approach that utilizes a large available source database combined with a location-specific (or site-specific) source database.

FUTURE DIRECTIONS

In general, future studies (not just at these three impaired watersheds) would be useful in the following areas:

- BST studies would benefit from the development of standard protocols for sampling and data interpretation, including the total number of isolates to source-track in a stream system, the number of isolates to source-track from each water sample, and the design and frequency of sampling. In developing these protocols, the different objectives of the BST studies must be considered.
- The transport mechanisms by which bacteria can be routed into a stream should be identified.
- After the transport mechanisms have been identified and source-management practices have been implemented, the capacity of these practices to reduce source inputs to streams should be evaluated.
- BST data should provide support and guidance for the production of more defendable and scientifically rigorous watershed models. Incorporation of these source-tracking data into watershed-management strategies should result in the selection of more efficient source-reduction scenarios for improving water quality.
- Presently, BST studies are probably too expensive to be performed in all impaired stream systems. Cost-effective strategies are needed for generating bacteria-source information that can be applied to the large number of watersheds for which fecal coliform watershed models still must be developed.

SUMMARY AND CONCLUSIONS

The U.S. Geological Survey, in cooperation with the Virginia Department of Environmental Quality, Virginia Department of Conservation and Recreation, and Fairfax County, began a 3-year study in 1999 to perform bacterial source tracking (BST) on three streams in Virginia. The three streams selected for this study were Accotink Creek, Christians Creek, and Blacks Run, because they represented a range of different land-use practices (urban, agricultural, and mixed urban/agricultural, respectively) and potential fecal coliform sources. The Virginia Department of Environmental Quality classified these three streams as impaired by fecal coliform bacteria because of violations of the of the State’s water-quality standard (1,000 col/100mL). This study was performed to demonstrate the field application of BST technology and to identify the sources of fecal coliform bacteria in these three impaired streams. The three streams were sampled over a period of 20 months (March 1999–October 2000) and over a wide range of hydrological conditions. The ribotyping technique was used to identify the sources of the fecal coliform bacteria.

This study demonstrated the utility of BST technology and provided an enhanced understanding of the fecal coliform concentrations and sources that impaired the Accotink Creek, Blacks Run, and Christians Creek watersheds in Virginia. The major findings and conclusions of this study are:

- Fecal coliform concentrations were lowest during periods of base flow (typically 200–2,000 col/100mL) and increased by 3–4 orders of magnitude during storm events (as high as 700,000 col/100mL).
- Multiple linear regression models can be developed to predict fecal coliform bacteria concentrations in these streams as a function of water-quality parameters (turbidity, pH, water temperature, and dissolved oxygen concentration).
- The major contributors of fecal coliform bacteria in each watershed, in order of importance, were:
  - **Accotink Creek:** geese, humans, dogs, ducks, cats, seagulls, and raccoons.
  - **Blacks Run:** cattle, poultry, humans, dogs, and cats.
  - **Christians Creek:** poultry, cattle, humans, dogs, horses, and deer.
- Identified bacteria sources were related to the land-use practices within each watershed.
- For Christians Creek and Blacks Run, seasonal patterns were present in the contributions of *E. coli* from cattle and poultry sources. Cattle sources were more prevalent during the warm months (April–September), whereas poultry sources were more prevalent during the cool months (October–March).
- There were only minor differences in the distribution of bacteria sources between low-flow periods and storm-flow periods.
- A coupled approach that utilized both a large available source library and a smaller, location-specific source library provided the most success in identifying unknown *E. coli* isolates.
- Future studies would benefit from the development of more cost-effective, standardized protocols for BST techniques, sampling strategies, and data analyses.

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Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
			Accotink Creek		
04/13/99	1000	WAC06	23470	Feline	E, V
04/13/99	1000	WAC06	23471	Goose	V
04/13/99	1005	WAC07	23473	Opossum	n.r.
04/13/99	1005	WAC07	23472	–	n.a.
04/13/99	1010	WAC08	23474	Avian	E
04/13/99	1010	WAC08	23475	Avian	E
04/13/99	1015	WAC09	23477	Dog	E
04/13/99	1015	WAC09	23476	–	n.a.
04/13/99	1020	WAC10	23478	Dog	E
04/13/99	1020	WAC10	23479	Dog	E
04/13/99	1025	WAC11	23480	–	n.a.
04/13/99	1025	WAC11	23481	–	n.a.
04/13/99	1030	WAC12	23482	–	n.a.
04/13/99	1030	WAC12	23483	–	n.a.
04/13/99	1035	WAC13	23485	Dog	E
04/13/99	1035	WAC13	23484	–	n.a.
05/24/99	1115	WAC15	23787	Raccoon	E
05/24/99	1115	WAC15	23788	Raccoon	E
05/24/99	1130	WAC16	23790	Human	E
05/24/99	1130	WAC16	23789	–	n.a.
05/24/99	1145	WAC17	23792	Human	E
05/24/99	1145	WAC17	23791	Human	E
05/24/99	1200	WAC18	23794	Human	E
05/24/99	1200	WAC18	23793	–	n.a.
05/24/99	1230	WAC19	23796	Feline	E
05/24/99	1230	WAC19	23795	Raccoon	E
05/24/99	1300	WAC20	23798	Duck	E
05/24/99	1300	WAC20	23797	–	n.a.
05/24/99	1330	WAC21	23799	Dog	E
05/24/99	1530	WAC22	23800	Dog	E
05/24/99	1530	WAC22	23801	–	n.a.
05/24/99	1730	WAC23	23802	Goose	V
05/24/99	1730	WAC23	23803	Goose	V
05/24/99	1900	WAC24	23805	Duck	E
05/24/99	1900	WAC24	23804	Transient	n.r.
05/27/99	1115	WAC25	23869	Dog	E
05/27/99	1120	WAC26	23870	Dog	E
05/27/99	1125	WAC27	23872	Canine	n.r.
05/27/99	1125	WAC27	23871	Fox	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
05/27/99	1130	WAC28	23874	Goose	E
05/27/99	1130	WAC28	23873	–	n.a.
05/27/99	1135	WAC29	23876	Dog	E
05/27/99	1135	WAC29	23875	–	n.a.
05/27/99	1140	WAC30	23878	Human	E, V
05/27/99	1140	WAC30	23877	Sea Gull	E
05/27/99	1145	WAC31	23879	Human	E, V
05/27/99	1150	WAC32	23880	–	n.a.
05/27/99	1150	WAC32	23881	–	n.a.
07/07/99	1100	WAC33	24353	Dog	E
07/07/99	1100	WAC33	24354	Human	E
07/07/99	1105	WAC34	24356	Duck	E
07/07/99	1105	WAC34	24355	–	n.a.
07/07/99	1110	WAC35	24358	Feline	E
07/07/99	1110	WAC35	24357	–	n.a.
07/07/99	1115	WAC36	24359	Avian	E
07/07/99	1115	WAC36	24360	Human	E
07/07/99	1120	WAC37	24361	Avian	E
07/07/99	1120	WAC37	24362	Dog	E
07/07/99	1125	WAC38	24363	Avian	E
07/07/99	1125	WAC38	24364	Avian	E
07/07/99	1125	WAC38	24365	Feline	E
07/07/99	1130	WAC39	24368	Goose	V
07/07/99	1130	WAC39	24367	Human	E, V
07/07/99	1135	WAC40	24366	Human	E, V
07/07/99	1135	WAC40	24369	–	n.a.
07/07/99	1135	WAC40	24370	–	n.a.
08/14/99	1640	WAC47	24830	Goose	E
08/14/99	1640	WAC47	24828	Rodent	E
08/14/99	1640	WAC47	24829	–	n.a.
08/14/99	1745	WAC48	24831	Avian	E
08/14/99	1745	WAC48	24832	Avian	E
08/14/99	1745	WAC48	24833	Goose	E, V
08/14/99	1915	WAC49	24834	Duck	E
08/14/99	1915	WAC49	24835	Human	E
08/14/99	1945	WAC50	24836	Duck	E
08/14/99	2000	WAC51	24838	Dog	E
08/14/99	2000	WAC51	24837	Human	E
08/14/99	2000	WAC51	24839	Raccoon	E
08/14/99	2010	WAC52	24842	Dog	E
08/14/99	2010	WAC52	24840	Dog	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
08/14/99	2010	WAC52	24841	Goose	E, V
08/14/99	2025	WAC53	24845	Bovine	E
08/14/99	2025	WAC53	24844	Duck	E
08/14/99	2025	WAC53	24843	Feline	E, V
08/14/99	2100	WAC54	24847	Feline	E, V
08/14/99	2100	WAC54	24848	Goose	E
08/14/99	2100	WAC54	24846	–	n.a.
08/14/99	2145	WAC55	24849	Avian	n.r.
08/14/99	2145	WAC55	24850	Rodent	E
08/14/99	2145	WAC55	24851	Rodent	E
08/15/99	15	WAC56	24852	Goose	V
08/15/99	15	WAC56	24853	–	n.a.
08/15/99	15	WAC56	24854	–	n.a.
08/17/99	1005	WAC59	24855	Goose	E, V
08/17/99	1010	WAC60	24856	–	n.a.
08/17/99	1010	WAC60	24857	–	n.a.
08/17/99	1010	WAC60	24858	–	n.a.
08/17/99	1020	WAC62	24859	Duck	E, V
08/17/99	1020	WAC62	24860	–	n.a.
08/17/99	1030	WAC64	24861	Goose	E, V
08/17/99	1030	WAC64	24862	Human	E
09/10/99	2055	WAC65	25306	Avian	E
09/10/99	2055	WAC65	25305	Dog	E
09/10/99	2137	WAC66	25308	Dog	n.r.
09/10/99	2137	WAC66	25307	Human	V
09/10/99	2330	WAC67	25310	Rodent	E
09/10/99	2330	WAC67	25311	Rodent	E
09/10/99	2330	WAC67	25309	–	n.a.
09/10/99	2330	WAC67	25312	–	n.a.
09/10/99	15	WAC68	25315	Digested Sludge	E
09/10/99	15	WAC68	25314	Feline	E, V
09/10/99	15	WAC68	25313	Human	E
09/10/99	15	WAC68	25316	–	n.a.
09/10/99	100	WAC69	25319	Goose	E
09/10/99	100	WAC69	25320	Goose	E
09/10/99	100	WAC69	25317	Sludge	n.r.
09/10/99	100	WAC69	25318	–	n.a.
09/10/99	124	WAC71	25321	Dog	E
09/10/99	124	WAC71	25323	Dog	E
09/10/99	124	WAC71	25324	Duck	E
09/10/99	124	WAC71	25322	Human	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
09/10/99	124	WAC71	25325	Human	E
09/10/99	154	WAC72	25326	Goose	E, V
09/10/99	154	WAC72	25327	Human	E
09/10/99	154	WAC72	25328	Human	E
09/10/99	320	WAC73	25330	Feline	V
09/10/99	320	WAC73	25331	Feline	V
09/10/99	320	WAC73	25329	–	n.a.
09/10/99	320	WAC73	25332	–	n.a.
09/10/99	603	WAC74	25333	Goose	E
09/15/99	640	WAC75	25707	Human	E
09/15/99	640	WAC75	25704	Goose	E
09/15/99	640	WAC75	25706	Human	E
09/15/99	640	WAC75	25705	–	n.a.
09/15/99	900	WAC76	25710	Dog	E
09/15/99	900	WAC76	25708	Duck	E, V
09/15/99	900	WAC76	25709	–	n.a.
09/15/99	945	WAC77	25713	Sea Gull	E
09/15/99	945	WAC77	25711	–	n.a.
09/15/99	945	WAC77	25712	–	n.a.
09/15/99	945	WAC77	25714	–	n.a.
09/15/99	1115	WAC78	25715	Bovine	E
09/15/99	1115	WAC78	25717	Goose	E, V
09/15/99	1115	WAC78	25718	Goose	E, V
09/15/99	1115	WAC78	25716	–	n.a.
09/15/99	1230	WAC79	25722	Dog	E
09/15/99	1230	WAC79	25721	Human	E, V
09/15/99	1230	WAC79	25719	Muskrat	V
09/15/99	1230	WAC79	25720	Muskrat	V
09/15/99	1315	WAC80	25725	Dog	E
09/15/99	1315	WAC80	25723	–	n.a.
09/15/99	1315	WAC80	25724	–	n.a.
09/15/99	1315	WAC80	25726	–	n.a.
09/15/99	1430	WAC81	25729	Septage	n.r.
09/15/99	1430	WAC81	25727	–	n.a.
09/15/99	1430	WAC81	25728	–	n.a.
09/15/99	1545	WAC82	25733	Goose	E
09/15/99	1545	WAC82	25732	Human	E
09/15/99	1545	WAC82	25731	Human	E, V
09/15/99	1545	WAC82	25730	Sea Gull	E
09/15/99	1730	WAC83	25734	–	n.a.
09/15/99	2100	WAC84	25735	Coyote	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
09/15/99	2100	WAC84	25736	–	n.a.
09/16/99	900	WAC85	25738	combined sewer overflow	E
09/16/99	900	WAC85	25737	Raccoon	E
09/16/99	900	WAC85	25739	–	n.a.
09/27/99	1105	WAC86	25847	Rodent	E
09/27/99	1105	WAC86	25849	Sea Gull	E
09/27/99	1105	WAC86	25848	–	n.a.
09/27/99	1110	WAC87	25851	Raccoon	E
09/27/99	1110	WAC87	25850	–	n.a.
09/27/99	1110	WAC87	25852	–	n.a.
09/27/99	1110	WAC87	25853	–	n.a.
09/27/99	1115	WAC88	25855	Avian	E
09/27/99	1115	WAC88	25854	Dog	E, V
09/27/99	1115	WAC88	25856	–	n.a.
09/27/99	1115	WAC88	25857	–	n.a.
09/27/99	1120	WAC89	25858	Bovine	E, V
09/27/99	1120	WAC89	25861	Goose	E
09/27/99	1120	WAC89	25859	Goose	E, V
09/27/99	1120	WAC89	25860	–	n.a.
09/27/99	1125	WAC90	25863	Dog	E
09/27/99	1125	WAC90	25865	Dog	E
09/27/99	1125	WAC90	25862	–	n.a.
09/27/99	1125	WAC90	25864	–	n.a.
09/27/99	1130	WAC91	25868	Avian	E
09/27/99	1130	WAC91	25869	Avian	E
09/27/99	1130	WAC91	25867	Duck	E
09/27/99	1130	WAC91	25866	Septage	n.r.
09/27/99	1135	WAC92	25870	Avian	E
09/27/99	1135	WAC92	25871	Avian	E
09/27/99	1135	WAC92	25873	Dog	n.r.
09/27/99	1135	WAC92	25872	–	n.a.
09/27/99	1140	WAC93	25876	Avian	E
09/27/99	1140	WAC93	25877	Raccoon	E, V
09/27/99	1140	WAC93	25874	–	n.a.
09/27/99	1140	WAC93	25875	–	n.a.
09/27/99	1140	WAC93B	25880	Avian	E
09/27/99	1140	WAC93B	25878	–	n.a.
09/27/99	1140	WAC93B	25879	–	n.a.
09/27/99	1140	WAC93B	25881	–	n.a.
09/27/99	1140	WAC93C	25882	Dog	E
09/27/99	1140	WAC93C	25883	Raccoon	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

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Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
09/27/99	1140	WAC93C	25884	–	n.a.
09/27/99	1140	WAC93C	25885	–	n.a.
11/10/99	940	WAC94	26511	–	n.a.
11/10/99	940	WAC94	26512	Human	E
11/10/99	940	WAC94	26513	Septage	n.r.
11/10/99	940	WAC94	26514	Bovine	E, V
11/10/99	945	WAC95	26515	Human	E
11/10/99	945	WAC95	26516	Goose	E, V
11/10/99	945	WAC95	26517	Feline	E
11/10/99	945	WAC95	26518	–	n.a.
11/10/99	950	WAC96	26519	Goose	E, V
11/10/99	950	WAC96	26520	Goose	E
11/10/99	950	WAC96	26521	Human	E
11/10/99	950	WAC96	26522	–	n.a.
11/10/99	955	WAC97	26523	Human	E
11/10/99	955	WAC97	26524	Duck	E
11/10/99	955	WAC97	26525	–	n.a.
11/10/99	955	WAC97	26526	Bovine	E, V
11/10/99	1000	WAC98	26527	Goose	E, V
11/10/99	1000	WAC98	26528	Raccoon	E
11/10/99	1000	WAC98	26529	–	n.a.
11/10/99	1000	WAC98	26530	Raccoon	E
11/10/99	1005	WAC99	26531	Sea Gull	n.r.
11/10/99	1005	WAC99	26532	Rabbit	E
11/10/99	1005	WAC99	26533	Rabbit	E
11/10/99	1005	WAC99	26534	Deer	E
11/10/99	1010	WAC100	26535	Deer	E
11/10/99	1010	WAC100	26536	Dog	V
11/10/99	1010	WAC100	26537	Human	E
11/10/99	1010	WAC100	26538	–	n.a.
11/10/99	1015	WAC101	26539	–	n.a.
11/10/99	1015	WAC101	26540	Human	E, V
11/10/99	1015	WAC101	26541	Human	E, V
11/10/99	1015	WAC101	26542	Feline	E
11/10/99	1015	WAC101B	26543	Goose	E
11/10/99	1015	WAC101B	26544	Dog	E
11/10/99	1015	WAC101B	26545	–	n.a.
11/10/99	1015	WAC101B	26546	–	n.a.
11/10/99	1015	WAC101B	26547	Avian	E
11/10/99	1015	WAC101C	26548	Avian	n.r.
11/10/99	1015	WAC101C	26549	Avian	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/10/99	1015	WAC101C	26550	Feline	E
11/10/99	1015	WAC101C	26551	Dog	E
12/21/99	1215	WAC102	26864	Avian	E
12/21/99	1215	WAC102	26865	Goose	n.r.
12/21/99	1215	WAC102	26866	Goose	n.r.
12/21/99	1220	WAC103	26868	Sea Gull	E
12/21/99	1220	WAC103	26870	Sea Gull	E
12/21/99	1220	WAC103	26867	–	n.a.
12/21/99	1220	WAC103	26869	–	n.a.
12/21/99	1225	WAC104	26874	Digested Sludge	E
12/21/99	1225	WAC104	26873	Goose	E
12/21/99	1225	WAC104	26871	–	n.a.
12/21/99	1225	WAC104	26872	–	n.a.
12/21/99	1230	WAC105	26877	Goose	E
12/21/99	1230	WAC105	26878	Goose	V
12/21/99	1230	WAC105	26875	Human	E
12/21/99	1230	WAC105	26876	Sheep	V
12/21/99	1235	WAC106	26881	Goose	V
12/21/99	1235	WAC106	26879	–	n.a.
12/21/99	1235	WAC106	26880	–	n.a.
12/21/99	1235	WAC106	26882	–	n.a.
12/21/99	1240	WAC107	26883	Human	E
12/21/99	1240	WAC107	26884	Sea Gull	E
12/21/99	1240	WAC107	26886	Sea Gull	E
12/21/99	1240	WAC107	26885	–	n.a.
12/21/99	1245	WAC108	26890	Avian	n.r.
12/21/99	1245	WAC108	26887	Canine	E
12/21/99	1245	WAC108	26888	Goose	V
12/21/99	1245	WAC108	26889	–	n.a.
12/21/99	1250	WAC109	26892	Dog	E
12/21/99	1250	WAC109	26893	Duck	E
12/21/99	1250	WAC109	26891	Human	V
12/21/99	1250	WAC109	26894	Human	E, V
12/21/99	1250	WAC109B	26896	Feline	n.r.
12/21/99	1250	WAC109B	26897	Goose	E
12/21/99	1250	WAC109B	26895	–	n.a.
12/21/99	1250	WAC109B	26898	–	n.a.
12/21/99	1250	WAC109C	26899	Dog	n.r.
12/21/99	1250	WAC109C	26900	Goose	E, V
03/24/00	1315	WAC111	27877	Human	n.r.
03/24/00	1315	WAC111	27875	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
03/24/00	1315	WAC111	27876	–	n.a.
03/24/00	1320	WAC112	27878	Avian	E
03/24/00	1320	WAC112	27879	Human	n.r.
03/24/00	1320	WAC112	27880	–	n.a.
03/24/00	1325	WAC113	27882	Dog	E
03/24/00	1325	WAC113	27883	Human	V
03/24/00	1325	WAC113	27881	Human	n.r.
03/24/00	1330	WAC114	27884	Human	V
03/24/00	1330	WAC114	27886	Transient	n.r.
03/24/00	1330	WAC114	27885	–	n.a.
03/24/00	1330	WAC114B	27887	Avian	E
03/24/00	1330	WAC114B	27888	Bear	E
03/24/00	1330	WAC114B	27889	–	n.a.
03/24/00	1330	WAC114C	27891	Rodent	E
03/24/00	1330	WAC114C	27892	Rodent	E
03/24/00	1330	WAC114C	27890	–	n.a.
05/03/00	1315	WAC115	29752	Avian	E
05/03/00	1315	WAC115	29753	Avian	E
05/03/00	1315	WAC115	29751	–	n.a.
05/03/00	1320	WAC116	29754	Human	E
05/03/00	1320	WAC116	29756	Human	V
05/03/00	1320	WAC116	29755	Swan	V
05/03/00	1325	WAC117	29757	Human	V
05/03/00	1325	WAC117	29758	Human	E
05/03/00	1325	WAC117	29759	Rodent	E
05/03/00	1330	WAC118	29762	Avian	E, V
05/03/00	1330	WAC118	29760	Human	V
05/03/00	1330	WAC118	29761	–	n.a.
05/03/00	1330	WAC118B	29764	Raccoon	V
05/03/00	1330	WAC118B	29763	–	n.a.
05/03/00	1330	WAC118B	29765	–	n.a.
05/03/00	1330	WAC118C	29767	Avian	E
05/03/00	1330	WAC118C	29766	–	n.a.
05/03/00	1330	WAC118C	29768	–	n.a.
05/31/00	1100	WAC119	31168	Avian	n.r.
05/31/00	1100	WAC119	31169	Duck	E
05/31/00	1100	WAC119	31170	–	n.a.
05/31/00	1105	WAC120	31172	Avian	E
05/31/00	1105	WAC120	31173	Human	V
05/31/00	1105	WAC120	31171	Raccoon	E
05/31/00	1110	WAC121	31175	Avian	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
05/31/00	1110	WAC121	31174	Human	E
05/31/00	1110	WAC121	31176	Raccoon	E
05/31/00	1115	WAC122	31179	Goose	V
05/31/00	1115	WAC122	31177	–	n.a.
05/31/00	1115	WAC122	31178	–	n.a.
05/31/00	1115	WAC122B	31181	Bovine	E, V
05/31/00	1115	WAC122B	31182	Feline	E
05/31/00	1115	WAC122B	31180	–	n.a.
05/31/00	1115	WAC122C	31183	Feline	E
05/31/00	1115	WAC122C	31184	–	n.a.
05/31/00	1115	WAC122C	31185	–	n.a.
06/05/00	2000	WAC123	31502	Dog	E
06/05/00	2000	WAC123	31501	Sea Gull	E
06/06/00	815	WAC124	31504	Duck	E
06/06/00	815	WAC124	31505	Duck	E
06/06/00	815	WAC124	31503	–	n.a.
06/06/00	935	WAC125	31507	Deer	E
06/06/00	935	WAC125	31508	Deer	E
06/06/00	935	WAC125	31506	–	n.a.
06/06/00	1000	WAC126	31509	Goose	n.r.
06/06/00	1000	WAC126	31510	Goose	n.r.
06/06/00	1000	WAC126	31511	–	n.a.
06/06/00	1015	WAC127	31513	Dog	E
06/06/00	1015	WAC127	31514	Human	E, V
06/06/00	1015	WAC127	31512	–	n.a.
06/06/00	1030	WAC128	31516	Ground Hog	V
06/06/00	1030	WAC128	31515	Rabbit	E
06/06/00	1030	WAC128	31517	–	n.a.
06/06/00	1045	WAC129	31519	Bovine	E
06/06/00	1045	WAC129	31518	Feline	E
06/06/00	1045	WAC129	31520	Human	E
06/06/00	1050	WAC130	31521	–	n.a.
06/06/00	1050	WAC130	31522	–	n.a.
06/06/00	1050	WAC130	31523	–	n.a.
06/06/00	1130	WAC131	31526	Human	E, V
06/06/00	1130	WAC131	31524	Raccoon	E
06/06/00	1130	WAC131	31525	Raccoon	E
06/06/00	1310	WAC132	31528	Avian	n.r.
06/06/00	1310	WAC132	31529	Avian	n.r.
06/06/00	1310	WAC132	31527	–	n.a.
06/06/00	2037	WAC133	31531	Avian	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
06/06/00	2037	WAC133	31532	Avian	E
06/06/00	2037	WAC133	31530	–	n.a.
07/11/00	1100	WAC134	33258	Duck	E, V
07/11/00	1100	WAC134	33257	Feline	E
07/11/00	1100	WAC134	33259	Feline	E
07/11/00	1105	WAC135	33260	Feline	E
07/11/00	1105	WAC135	33261	Human	E
07/11/00	1105	WAC135	33262	Human	E
07/11/00	1110	WAC136	33263	Poultry	n.r.
07/11/00	1110	WAC136	33264	–	n.a.
07/11/00	1110	WAC136	33265	–	n.a.
07/11/00	1115	WAC137	33266	Avian	n.r.
07/11/00	1115	WAC137	33267	Goose	V
07/11/00	1115	WAC137	33268	Goose	V
07/11/00	1115	WAC137B	33270	Horse	E
07/11/00	1115	WAC137B	33269	Rodent	E
07/11/00	1115	WAC137B	33271	–	n.a.
07/11/00	1115	WAC137C	33273	Feline	E
07/11/00	1115	WAC137C	33272	Human	E
07/11/00	1115	WAC137C	33274	Human	V
08/10/00	745	WAC143	34687	Goose	E, V
08/10/00	745	WAC143	34688	Goose	E, V
08/10/00	745	WAC143	34686	–	n.a.
08/10/00	750	WAC144	34691	Human	V
08/10/00	750	WAC144	34689	–	n.a.
08/10/00	750	WAC144	34690	–	n.a.
08/10/00	750	WAC145	34692	–	n.a.
08/10/00	750	WAC145	34693	–	n.a.
08/10/00	750	WAC145	34694	–	n.a.
08/10/00	755	WAC146	34695	Human	E, V
08/10/00	755	WAC146	34696	–	n.a.
08/10/00	755	WAC146	34697	–	n.a.
08/10/00	800	WAC147	34699	Avian	E
08/10/00	800	WAC147	34700	Avian	E
08/10/00	800	WAC147	34698	–	n.a.
08/10/00	800	WAC148	34703	Dog	E
08/10/00	800	WAC148	34702	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
			Blacks Run		
04/27/99	1430	WBR06	23551	Human	E
04/27/99	1430	WBR06	23552	Raccoon	V
04/27/99	1435	WBR07	23554	Bovine	E, V
04/27/99	1435	WBR07	23553	Dog	E
04/27/99	1440	WBR08	23556	Human	E
04/27/99	1440	WBR08	23555	–	n.a.
04/27/99	1445	WBR09	23557	Bovine	E
04/27/99	1445	WBR09	23558	–	n.a.
04/27/99	1450	WBR10	23559	Bovine	E
04/27/99	1450	WBR10	23560	Human	E
04/27/99	1455	WBR11	23561	Bovine	E
04/27/99	1455	WBR11	23562	–	n.a.
04/27/99	1500	WBR12	23564	Dog	E, V
04/27/99	1500	WBR12	23563	–	n.a.
04/27/99	1505	WBR13	23565	Avian	E
04/27/99	1505	WBR13	23566	–	n.a.
05/08/99	300	WBR14	23674	Bovine	E
05/08/99	300	WBR14	23675	Bovine	E
05/08/99	345	WBR15	23676	Bovine	E
05/08/99	345	WBR15	23677	Sea Gull	E
05/08/99	445	WBR16	23678	Feline	E, V
05/08/99	445	WBR16	23679	Sea Gull	E
05/08/99	545	WBR17	23681	Bovine	V
05/08/99	545	WBR17	23680	Poultry	E
05/08/99	745	WBR18	23683	Horse	E
05/08/99	745	WBR18	23682	Human	E
05/08/99	1015	WBR19	23684	–	n.a.
05/08/99	1015	WBR19	23685	–	n.a.
05/08/99	1115	WBR20	23686	–	n.a.
05/08/99	1115	WBR20	23687	–	n.a.
05/08/99	1345	WBR21	23688	Dog	E
05/08/99	1345	WBR21	23689	Horse	E
05/08/99	1445	WBR22	23690	Bovine	E, V
05/08/99	1445	WBR22	23691	–	n.a.
05/08/99	1645	WBR23	23692	Bovine	E
05/08/99	1645	WBR23	23693	–	n.a.
06/09/99	1425	WBR24	24128	Dog	E
06/09/99	1425	WBR24	24129	–	n.a.
06/09/99	1430	WBR25	24131	Bovine	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
06/09/99	1430	WBR25	24130	Poultry	E
06/09/99	1435	WBR26	24133	Human	E
06/09/99	1435	WBR26	24132	–	n.a.
06/09/99	1440	WBR27	24135	Bovine	E, V
06/09/99	1440	WBR27	24134	Dog	E
06/09/99	1445	WBR28	24136	Bovine	V
06/09/99	1445	WBR28	24137	Duck	V
06/09/99	1450	WBR29	24139	Dog	E
06/09/99	1450	WBR29	24138	–	n.a.
06/09/99	1455	WBR30	24140	Feline	E
06/09/99	1455	WBR30	24141	Poultry	E, V
06/09/99	1500	WBR31	24143	Human	E
06/09/99	1500	WBR31	24142	Poultry	E, V
07/20/99	925	WBR32	24615	Human	V
07/20/99	925	WBR32	24616	Human	V
07/20/99	930	WBR33	24617	–	n.a.
07/20/99	930	WBR33	24618	Bovine	n.r.
07/20/99	935	WBR34	24619	Bovine	n.r.
07/20/99	935	WBR34	24620	–	n.a.
07/20/99	940	WBR35	24621	Bovine	E
07/20/99	940	WBR35	24622	Bovine	E
07/20/99	945	WBR36	24623	Bovine	E
07/20/99	945	WBR36	24624	–	n.a.
07/20/99	950	WBR37	24625	Human	E
07/20/99	950	WBR37	24626	Bovine	E, V
07/20/99	955	WBR38	24627	Avian	E
07/20/99	955	WBR38	24628	–	n.a.
07/20/99	920	WBR39	24629	Bovine	E
07/20/99	920	WBR39	24630	–	n.a.
08/25/99	1250	WBR51	24892	–	n.a.
08/25/99	1250	WBR51	24893	–	n.a.
08/25/99	1250	WBR51	24894	–	n.a.
08/25/99	1255	WBR52	24896	Bovine	E
08/25/99	1255	WBR52	24898	Bovine	E, V
08/25/99	1255	WBR52	24895	Poultry	V
08/25/99	1255	WBR52	24897	–	n.a.
08/25/99	1300	WBR53	24900	Bovine	E
08/25/99	1300	WBR53	24899	–	n.a.
08/25/99	1300	WBR53	24901	–	n.a.
08/25/99	1305	WBR54	24903	Feline	E
08/25/99	1305	WBR54	24902	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
08/25/99	1305	WBR54	24904	–	n.a.
08/25/99	1310	WBR55	24906	Human	E
08/25/99	1310	WBR55	24907	Human	E
08/25/99	1310	WBR55	24905	–	n.a.
08/25/99	1315	WBR56	24909	Human	E
08/25/99	1315	WBR56	24908	–	n.a.
08/25/99	1315	WBR56	24910	–	n.a.
08/25/99	1320	WBR57	24911	Poultry	V
08/25/99	1320	WBR57	24912	–	n.a.
08/25/99	1320	WBR57	24913	–	n.a.
08/25/99	1325	WBR58	24915	Poultry	V
08/25/99	1325	WBR58	24914	–	n.a.
08/25/99	1325	WBR58	24916	–	n.a.
09/15/99	1845	WBR59	25741	Bovine	E
09/15/99	1845	WBR59	25740	Poultry	E
09/15/99	1845	WBR59	25742	–	n.a.
09/15/99	1845	WBR59	25743	–	n.a.
09/15/99	2127	WBR60	25744	–	n.a.
09/15/99	2127	WBR60	25745	–	n.a.
09/15/99	2127	WBR60	25746	–	n.a.
09/15/99	2127	WBR60	25747	–	n.a.
09/15/99	542	WBR61	25749	Bovine	E
09/15/99	542	WBR61	25748	–	n.a.
09/15/99	542	WBR61	25750	–	n.a.
09/15/99	542	WBR61	25751	–	n.a.
09/15/99	610	WBR62	25752	Avian	E
09/15/99	610	WBR62	25753	–	n.a.
09/15/99	610	WBR62	25754	–	n.a.
09/15/99	610	WBR62	25755	–	n.a.
09/15/99	745	WBR63	25758	Bovine	E
09/15/99	745	WBR63	25756	Poultry	n.r.
09/15/99	745	WBR63	25757	–	n.a.
09/15/99	745	WBR63	25759	–	n.a.
09/15/99	916	WBR64	25762	Bovine	E, V
09/15/99	916	WBR64	25760	Horse	E
09/15/99	916	WBR64	25761	Human	V
09/15/99	916	WBR64	25763	Poultry	E, V
09/15/99	1045	WBR65	25767	Human	n.r.
09/15/99	1045	WBR65	25765	Opossum	n.r.
09/15/99	1045	WBR65	25766	Poultry	V
09/15/99	1045	WBR65	25764	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
09/15/99	1340	WBR66	25770	Bovine	E
09/15/99	1340	WBR66	25768	Poultry	n.r.
09/15/99	1340	WBR66	25769	Raccoon	E
09/15/99	1340	WBR66	25771	–	n.a.
09/15/99	1830	WBR67	25773	Avian	E
09/15/99	1830	WBR67	25775	Deer	E
09/15/99	1830	WBR67	25774	Poultry	V
09/15/99	1830	WBR67	25772	Sheep	E
09/15/99	730	WBR68	25776	Avian	E
09/15/99	730	WBR68	25777	Avian	E
09/15/99	730	WBR68	25778	Bovine	E
09/15/99	730	WBR68	25779	Poultry	V
010/13/99	930	WBR69	26130	Avian	n.r.
010/13/99	930	WBR69	26131	Poultry	V
010/13/99	930	WBR69	26129	–	n.a.
010/13/99	930	WBR69	26132	–	n.a.
010/13/99	935	WBR70	26134	Avian	E
010/13/99	935	WBR70	26133	–	n.a.
010/13/99	935	WBR70	26135	–	n.a.
010/13/99	935	WBR70	26136	–	n.a.
010/13/99	940	WBR71	26139	Bovine	E, V
010/13/99	940	WBR71	26140	Bovine	E, V
010/13/99	940	WBR71	26138	Human	V
010/13/99	940	WBR71	26137	–	n.a.
010/13/99	945	WBR72	26143	Dog	E
010/13/99	945	WBR72	26144	Sanitary Sewer	E
010/13/99	945	WBR72	26141	–	n.a.
010/13/99	945	WBR72	26142	–	n.a.
010/13/99	950	WBR73	26147	Avian	n.r.
010/13/99	950	WBR73	26145	Cat	E
010/13/99	950	WBR73	26148	Raccoon	E
010/13/99	950	WBR73	26146	–	n.a.
010/13/99	955	WBR74	26151	Bovine	E
010/13/99	955	WBR74	26152	Bovine	E
010/13/99	955	WBR74	26149	–	n.a.
010/13/99	955	WBR74	26150	–	n.a.
010/13/99	1000	WBR75	26156	Avian	n.r.
010/13/99	1000	WBR75	26155	Turkey	E
010/13/99	1000	WBR75	26153	Turkey	V
010/13/99	1000	WBR75	26154	–	n.a.
010/13/99	1005	WBR76	26160	Bovine	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
010/13/99	1005	WBR76	26158	Horse	E
010/13/99	1005	WBR76	26157	–	n.a.
010/13/99	1005	WBR76	26159	–	n.a.
010/13/99	1005	WBR76B	26164	Bovine	V
010/13/99	1005	WBR76B	26161	Bovine	E
010/13/99	1005	WBR76B	26162	Bovine	E
010/13/99	1005	WBR76B	26163	Bovine	E
010/13/99	1005	WBR76C	26166	Avian	n.r.
010/13/99	1005	WBR76C	26165	Bovine	E
010/13/99	1005	WBR76C	26168	Feline	E
010/13/99	1005	WBR76C	26167	–	n.a.
11/03/99	15	WBR77	26444	Horse	E
11/03/99	15	WBR77	26441	Human	E
11/03/99	15	WBR77	26442	–	n.a.
11/03/99	15	WBR77	26443	–	n.a.
11/03/99	630	WBR78	26445	Bovine	E
11/03/99	630	WBR78	26447	Bovine	V
11/03/99	630	WBR78	26448	Dog	E
11/03/99	630	WBR78	26446	–	n.a.
11/03/99	1300	WBR79	26451	Coyote	E
11/03/99	1300	WBR79	26452	Poultry	V
11/03/99	1300	WBR79	26449	–	n.a.
11/03/99	1300	WBR79	26450	–	n.a.
11/03/99	1515	WBR80	26454	Bovine	V
11/03/99	1515	WBR80	26453	–	n.a.
11/03/99	1515	WBR80	26455	–	n.a.
11/03/99	1515	WBR80	26456	–	n.a.
11/03/99	1630	WBR81	26459	Bovine	V
11/03/99	1630	WBR81	26458	Bovine	E, V
11/03/99	1630	WBR81	26460	Bovine	E, V
11/03/99	1630	WBR81	26457	Dog	E
11/03/99	1645	WBR82	26463	Human	E
11/03/99	1645	WBR82	26464	Human	E
11/03/99	1645	WBR82	26461	–	n.a.
11/03/99	1645	WBR82	26462	–	n.a.
11/03/99	1700	WBR83	26465	Avian	E
11/03/99	1700	WBR83	26468	Bovine	E, V
11/03/99	1700	WBR83	26466	Poultry	V
11/03/99	1700	WBR83	26467	–	n.a.
11/03/99	1715	WBR84	26471	Bovine	E, V
11/03/99	1715	WBR84	26470	Human	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/03/99	1715	WBR84	26469	Human	V
11/03/99	1715	WBR84	26472	Poultry	V
11/03/99	1730	WBR85	26476	Bovine	E
11/03/99	1730	WBR85	26474	Dog	E, V
11/03/99	1730	WBR85	26475	Turkey	E, V
11/03/99	1730	WBR85	26473	–	n.a.
11/03/99	1845	WBR86	26479	Human	E
11/03/99	1845	WBR86	26480	Turkey	E, V
11/03/99	1845	WBR86	26477	–	n.a.
11/03/99	1845	WBR86	26478	–	n.a.
11/03/99	2230	WBR87	26483	Dog	E
11/03/99	2230	WBR87	26481	Poultry	E
11/03/99	2230	WBR87	26484	Turkey	E, V
11/03/99	2230	WBR87	26482	–	n.a.
11/03/99	1100	WBR88	26488	Human	V
11/03/99	1100	WBR88	26486	Poultry	E
11/03/99	1100	WBR88	26485	Sheep	V
11/03/99	1100	WBR88	26487	–	n.a.
11/22/99	1050	WBR89	26713	Bovine	V
11/22/99	1050	WBR89	26712	–	n.a.
11/22/99	1050	WBR89	26714	–	n.a.
11/22/99	1050	WBR89	26715	–	n.a.
11/22/99	1055	WBR90	26717	Bovine	V
11/22/99	1055	WBR90	26718	Bovine	E, V
11/22/99	1055	WBR90	26716	Feline	E
11/22/99	1055	WBR90	26719	Poultry	V
11/22/99	1100	WBR91	26720	Bovine	V
11/22/99	1100	WBR91	26722	Poultry	V
11/22/99	1100	WBR91	26723	Poultry	V
11/22/99	1100	WBR91	26721	–	n.a.
11/22/99	1105	WBR92	26724	–	n.a.
11/22/99	1105	WBR92	26725	–	n.a.
11/22/99	1105	WBR92	26726	–	n.a.
11/22/99	1105	WBR92	26727	–	n.a.
11/22/99	1110	WBR93	26730	Avian	n.r.
11/22/99	1110	WBR93	26728	Avian	E
11/22/99	1110	WBR93	26729	Rodent	E
11/22/99	1110	WBR93	26731	Rodent	E
11/22/99	1115	WBR94	26732	Avian	E
11/22/99	1115	WBR94	26735	Bovine	E, V
11/22/99	1115	WBR94	26734	Bovine	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/22/99	1115	WBR94	26733	–	n.a.
11/22/99	1120	WBR95	26736	Bovine	E, V
11/22/99	1120	WBR95	26739	Bovine	E, V
11/22/99	1120	WBR95	26737	–	n.a.
11/22/99	1120	WBR95	26738	–	n.a.
11/22/99	1125	WBR96	26743	Avian	n.r.
11/22/99	1125	WBR96	26741	Bovine	E
11/22/99	1125	WBR96	26740	Dog	V
11/22/99	1125	WBR96	26742	–	n.a.
11/22/99	1125	WBR96B	26746	Avian	E
11/22/99	1125	WBR96B	26747	Avian	E
11/22/99	1125	WBR96B	26744	Human	E
11/22/99	1125	WBR96B	26745	–	n.a.
11/22/99	1125	WBR96C	26748	Avian	E
11/22/99	1125	WBR96C	26750	Avian	E
11/22/99	1125	WBR96C	26751	Horse	E
11/22/99	1125	WBR96C	26749	–	n.a.
01/04/00	1100	WBR97	26908	Poultry	V
01/04/00	1100	WBR97	26909	Avian	n.r.
01/04/00	1100	WBR97	26910	–	n.a.
01/04/00	1100	WBR97	26911	Dog	E
01/04/00	1105	WBR98	26912	Human	E, V
01/04/00	1105	WBR98	26913	Human	E
01/04/00	1105	WBR98	26914	Duck	E
01/04/00	1105	WBR98	26915	Feline	E
01/04/00	1110	WBR99	26916	–	n.a.
01/04/00	1110	WBR99	26917	Poultry	E
01/04/00	1110	WBR99	26918	–	n.a.
01/04/00	1110	WBR99	26919	Poultry	E
01/04/00	1115	WBR100	26920	–	n.a.
01/04/00	1115	WBR100	26921	Opossum	E
01/04/00	1115	WBR100	26922	Feline	E
01/04/00	1115	WBR100	26923	–	n.a.
01/04/00	1120	WBR101	26924	Deer	E
01/04/00	1120	WBR101	26925	Sheep	E
01/04/00	1120	WBR101	26926	Poultry	E
01/04/00	1120	WBR101	26927	Pig	E
01/04/00	1125	WBR102	26928	Poultry	E
01/04/00	1125	WBR102	26929	–	n.a.
01/04/00	1125	WBR102	26930	Turkey	E, V
01/04/00	1125	WBR102	26931	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
01/04/00	1130	WBR103	26932	Turkey	E, V
01/04/00	1130	WBR103	26933	Feline	E
01/04/00	1130	WBR103	26934	–	n.a.
01/04/00	1130	WBR103	26935	Feline	E
01/04/00	1135	WBR104	26936	Bovine	E
01/04/00	1135	WBR104	26937	Bovine	E
01/04/00	1135	WBR104	26938	Opossum	E
01/04/00	1135	WBR104	26939	Human	E
01/04/00	1135	WBR104B	26940	Bovine	E
01/04/00	1135	WBR104B	26941	Dog	E
01/04/00	1135	WBR104B	26942	Bovine	E
01/04/00	1135	WBR104B	26943	Opossum	n.r.
01/04/00	1135	WBR104C	26944	Human	E, V
01/04/00	1135	WBR104C	26945	Bovine	E
01/04/00	1135	WBR104C	26946	Human	E
01/04/00	1135	WBR104C	26947	–	n.a.
01/10/00	1003	WBR105	26972	Dog	E
01/10/00	1003	WBR105	26973	Avian	E
01/10/00	1003	WBR105	26974	Feline	E
01/10/00	1003	WBR105	26975	Bovine	E
01/10/00	1300	WBR108	26976	–	n.a.
01/10/00	1300	WBR108	26977	Avian	E
01/10/00	1300	WBR108	26978	–	n.a.
01/10/00	1300	WBR108	26979	Feline	E
01/10/00	1400	WBR111	26980	Bovine	E
01/10/00	1400	WBR111	26982	Bovine	E
01/10/00	1400	WBR111	26981	Coyote	E
01/10/00	1400	WBR111	26983	Pig	n.r.
01/10/00	1618	WBR112	26985	Avian	n.r.
01/10/00	1618	WBR112	26987	Bovine	E
01/10/00	1618	WBR112	26984	–	n.a.
01/10/00	1618	WBR112	26986	–	n.a.
01/10/00	1641	WBR113	26988	Bovine	E
01/10/00	1641	WBR113	26990	Bovine	E
01/10/00	1641	WBR113	26989	Coyote	E
01/10/00	1641	WBR113	26991	Duck	V
01/10/00	1700	WBR114	26992	Bovine	E, V
01/10/00	1700	WBR114	26995	Opossum	E
01/10/00	1700	WBR114	26993	Sheep	E
01/10/00	1700	WBR114	26994	–	n.a.
01/10/00	1715	WBR115	26998	Bovine	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
01/10/00	1715	WBR115	26997	Deer	E
01/10/00	1715	WBR115	26996	Feline	E
01/10/00	1715	WBR115	26999	Rodent	E
01/10/00	1900	WBR116	27002	Bovine	E
01/10/00	1900	WBR116	27003	Bovine	E
01/10/00	1900	WBR116	27000	–	n.a.
01/10/00	1900	WBR116	27001	–	n.a.
01/10/00	2205	WBR117	27007	Dog	E
01/10/00	2205	WBR117	27004	Human	n.r.
01/10/00	2205	WBR117	27006	Poultry	V
01/10/00	2205	WBR117	27005	–	n.a.
01/10/00	925	WBR118	27009	Bovine	V
01/10/00	925	WBR118	27008	Human	n.r.
01/10/00	925	WBR118	27010	–	n.a.
01/10/00	925	WBR118	27011	–	n.a.
01/10/00	1215	WBR119	27012	–	n.a.
01/10/00	1215	WBR119	27013	–	n.a.
01/10/00	1215	WBR119	27014	–	n.a.
01/10/00	1215	WBR119	27015	–	n.a.
03/30/00	1240	WBR121	27992	Human	E
03/30/00	1240	WBR121	27990	–	n.a.
03/30/00	1240	WBR121	27991	–	n.a.
03/30/00	1245	WBR122	27995	Avian	E
03/30/00	1245	WBR122	27993	Goose	E
03/30/00	1245	WBR122	27994	Human	E, V
03/30/00	1250	WBR123	27998	Poultry	n.r.
03/30/00	1250	WBR123	27997	Poultry	V
03/30/00	1250	WBR123	27996	–	n.a.
03/30/00	1250	WBR123B	28001	Avian	E
03/30/00	1250	WBR123B	27999	Human	E
03/30/00	1250	WBR123B	28000	Poultry	n.r.
03/30/00	1250	WBR123C	28003	Dog	E
03/30/00	1250	WBR123C	28002	Poultry	n.r.
03/30/00	1250	WBR123C	28004	–	n.a.
05/10/00	1205	WBR124	30228	Bovine	E
05/10/00	1205	WBR124	30226	Deer	E
05/10/00	1205	WBR124	30227	Deer	E
05/10/00	1210	WBR125	30229	–	n.a.
05/10/00	1210	WBR125	30230	–	n.a.
05/10/00	1210	WBR125	30231	–	n.a.
05/10/00	1215	WBR126	30233	Crow	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
05/10/00	1215	WBR126	30234	Dog	E
05/10/00	1215	WBR126	30232	–	n.a.
05/10/00	1215	WBR126B	30235	–	n.a.
05/10/00	1215	WBR126B	30236	–	n.a.
05/10/00	1215	WBR126B	30237	–	n.a.
05/10/00	1215	WBR126C	30239	Dog	E
05/10/00	1215	WBR126C	30238	–	n.a.
05/10/00	1215	WBR126C	30240	–	n.a.
06/14/00	1245	WBR127	31788	Avian	E
06/14/00	1245	WBR127	31786	Human	V
06/14/00	1245	WBR127	31787	–	n.a.
06/14/00	1250	WBR128	31789	Bovine	E
06/14/00	1250	WBR128	31790	Human	E
06/14/00	1250	WBR128	31791	Human	V
06/14/00	1255	WBR129	31792	Bovine	E
06/14/00	1255	WBR129	31793	Bovine	E
06/14/00	1255	WBR129	31794	Bovine	E
06/14/00	1255	WBR129B	31796	Human	E
06/14/00	1255	WBR129B	31797	Turkey	V
06/14/00	1255	WBR129B	31795	–	n.a.
06/14/00	1255	WBR129C	31799	Bovine	E, V
06/14/00	1255	WBR129C	31800	Goose	E
06/14/00	1255	WBR129C	31798	–	n.a.
06/14/00	1900	WBR130	31878	Dog	E
06/14/00	1900	WBR130	31877	–	n.a.
06/14/00	1900	WBR130	31879	–	n.a.
06/14/00	1957	WBR131	31880	Avian	n.r.
06/14/00	1957	WBR131	31882	Cat	E
06/14/00	1957	WBR131	31881	–	n.a.
06/14/00	2051	WBR132	31885	Poultry	E, V
06/14/00	2051	WBR132	31883	–	n.a.
06/14/00	2051	WBR132	31884	–	n.a.
06/14/00	2122	WBR133	31886	Canine	E
06/14/00	2122	WBR133	31887	–	n.a.
06/14/00	2122	WBR133	31888	–	n.a.
06/14/00	2143	WBR134	31889	Avian	E
06/14/00	2143	WBR134	31890	Avian	E
06/14/00	2143	WBR134	31891	–	n.a.
06/14/00	2215	WBR135	31894	Deer	E
06/14/00	2215	WBR135	31892	–	n.a.
06/14/00	2215	WBR135	31893	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
06/14/00	2235	WBR136	31895	Chicken	V
06/14/00	2235	WBR136	31896	–	n.a.
06/14/00	2235	WBR136	31897	–	n.a.
06/14/00	2345	WBR137	31898	Avian	E
06/14/00	2345	WBR137	31899	–	n.a.
06/14/00	2345	WBR137	31900	–	n.a.
06/15/00	220	WBR138	31903	Bovine	E
06/15/00	220	WBR138	31901	Rodent	E
06/15/00	220	WBR138	31902	–	n.a.
06/15/00	720	WBR139	31904	–	n.a.
07/18/00	1540	WBR140	33732	Raccoon	E, V
07/18/00	1540	WBR140	33733	Raccoon	E, V
07/18/00	1540	WBR140	33734	–	n.a.
07/18/00	1545	WBR141	33735	Bovine	V
07/18/00	1545	WBR141	33737	Feline	E
07/18/00	1545	WBR141	33736	Raccoon	E, V
07/18/00	1550	WBR142	33738	Bovine	E
07/18/00	1550	WBR142	33740	Raccoon	E, V
07/18/00	1550	WBR142	33739	–	n.a.
07/18/00	1550	WBR142B	33742	Avian	E
07/18/00	1550	WBR142B	33743	Raccoon	E, V
07/18/00	1550	WBR142B	33741	–	n.a.
07/18/00	1550	WBR142C	33744	–	n.a.
07/18/00	1550	WBR142C	33745	–	n.a.
07/18/00	1550	WBR142C	33746	–	n.a.
08/17/00	1135	WBR148	34950	Sludge	E
08/17/00	1135	WBR148	34949	–	n.a.
08/17/00	1135	WBR148	34951	–	n.a.
08/17/00	1140	WBR149	34952	Bovine	E
08/17/00	1140	WBR149	34954	Poultry	E, V
08/17/00	1140	WBR149	34953	–	n.a.
08/17/00	1140	WBR149B	34957	Human	E
08/17/00	1140	WBR149B	34955	Rabbit	E
08/17/00	1140	WBR149B	34956	–	n.a.
08/17/00	1145	WBR150	34958	Cat	E, V
08/17/00	1145	WBR150	34959	–	n.a.
08/17/00	1145	WBR150	34960	–	n.a.
08/17/00	1150	WBR151	34961	Goose	E
08/17/00	1150	WBR151	34962	–	n.a.
08/17/00	1150	WBR151	34963	–	n.a.
08/17/00	1150	WBR151B	34964	Avian	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
08/17/00	1150	WBR151B	34965	–	n.a.
08/17/00	1150	WBR151B	34966	–	n.a.
			Christians Creek		
5/5/99	910	WCC07	23597	Bovine	E
5/5/99	910	WCC07	23598	Sea Gull	n.r.
5/5/99	915	WCC08	23600	Bovine	E
5/5/99	915	WCC08	23599	Bovine	E, V
5/5/99	920	WCC09	23601	–	n.a.
5/5/99	920	WCC09	23602	–	n.a.
5/5/99	925	WCC10	23603	Bovine	V
5/5/99	925	WCC10	23604	–	n.a.
5/5/99	930	WCC11	23606	Feline	E, V
5/5/99	930	WCC11	23605	–	n.a.
5/5/99	935	WCC12	23608	Deer	E
5/5/99	935	WCC12	23607	Dog	E
5/5/99	940	WCC13	23610	–	n.a.
5/5/99	940	WCC13	23609	–	n.a.
5/5/99	945	WCC14	23612	Dog	E
5/5/99	945	WCC14	23611	Feline	E
6/16/99	1515	WCC16	24238	Rodent	E
6/16/99	1515	WCC16	24239	–	n.a.
6/16/99	715	WCC17	24240	–	n.a.
6/16/99	715	WCC17	24241	–	n.a.
6/16/99	830	WCC18	24243	Human	E
6/16/99	830	WCC18	24242	–	n.a.
6/16/99	935	WCC19	24245	Bovine	E
6/16/99	935	WCC19	24244	–	n.a.
6/16/99	945	WCC20	24246	Bovine	E, V
6/16/99	945	WCC20	24247	Bovine	E, V
6/16/99	1000	WCC21	24249	Bovine	E, V
6/16/99	1000	WCC21	24248	Bovine	E, V
6/16/99	1005	WCC22	24251	Bovine	E, V
6/16/99	1005	WCC22	24250	Bovine	E, V
6/16/99	1010	WCC23	24252	Bovine	E, V
6/16/99	1010	WCC23	24253	–	n.a.
7/28/99	845	WCC30	24684	Duck	E
7/28/99	845	WCC30	24685	Bovine	E, V
7/28/99	850	WCC31	24686	Horse	E
7/28/99	850	WCC31	24687	–	n.a.
7/28/99	855	WCC32	24688	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
7/28/99	855	WCC32	24689	Bovine	V
7/28/99	900	WCC33	24690	Human	E
7/28/99	900	WCC33	24691	Feline	E
7/28/99	905	WCC34	24692	Bovine	V
7/28/99	905	WCC34	24693	–	n.a.
7/28/99	910	WCC35	24694	–	n.a.
7/28/99	910	WCC35	24695	Human	E
7/28/99	915	WCC36	24696	Human	E
7/28/99	915	WCC36	24697	Poultry	V
7/28/99	920	WCC37	24698	Human	E, V
7/28/99	920	WCC37	24699	Avian	E
9/6/99	410	WCC39	25169	Dog	E, V
9/6/99	410	WCC39	25168	Turkey	E, V
9/6/99	410	WCC39	25167	Turkey	E, V
9/6/99	410	WCC39	25166	–	n.a.
9/6/99	825	WCC40	25172	Bovine	E
9/6/99	825	WCC40	25173	Bovine	V
9/6/99	825	WCC40	25170	Bovine	E
9/6/99	825	WCC40	25171	Duck	V
9/6/99	1220	WCC41	25174	Bovine	E
9/6/99	1220	WCC41	25176	Bovine	E, V
9/6/99	1220	WCC41	25177	Poultry	V
9/6/99	1220	WCC41	25175	–	n.a.
9/6/99	1650	WCC42	25178	Feline	E
9/6/99	1650	WCC42	25180	Horse	E
9/6/99	1650	WCC42	25181	Human	V
9/6/99	1650	WCC42	25179	–	n.a.
9/6/99	1815	WCC43	25183	Bovine	E
9/6/99	1815	WCC43	25182	Bovine	V
9/6/99	1815	WCC43	25184	Bovine	E, V
9/6/99	1900	WCC44	25186	Bovine	E
9/6/99	1900	WCC44	25185	Dog	V
9/6/99	1900	WCC44	25187	–	n.a.
9/6/99	1900	WCC44	25188	–	n.a.
9/6/99	1940	WCC45	25192	Avian	E
9/6/99	1940	WCC45	25191	Dog	E
9/6/99	1940	WCC45	25189	Human	E
9/6/99	1940	WCC45	25190	–	n.a.
9/6/99	2215	WCC46	25193	Avian	E
9/6/99	2215	WCC46	25194	Avian	E
9/6/99	2215	WCC46	25196	Poultry	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
9/6/99	2215	WCC46	25195	–	n.a.
9/6/99	50	WCC47	25197	Avian	E
9/6/99	50	WCC47	25199	Human	E
9/6/99	50	WCC47	25198	Sea Gull	E
9/6/99	50	WCC47	25200	–	n.a.
9/6/99	835	WCC48	25203	Bovine	E
9/6/99	835	WCC48	25204	Bovine	E
9/6/99	835	WCC48	25201	Bovine	E, V
9/6/99	835	WCC48	25202	Human	E
9/14/99	1110	WCC54	25336	–	n.a.
9/14/99	1110	WCC54	25337	–	n.a.
9/14/99	1110	WCC54	25335	–	n.a.
9/14/99	1110	WCC54	25334	–	n.a.
9/14/99	1115	WCC55	25338	Human	V
9/14/99	1115	WCC55	25339	–	n.a.
9/14/99	1115	WCC55	25340	–	n.a.
9/14/99	1115	WCC55	25341	–	n.a.
9/14/99	1120	WCC56	25343	Goose	n.r.
9/14/99	1120	WCC56	25345	–	n.a.
9/14/99	1120	WCC56	25342	–	n.a.
9/14/99	1120	WCC56	25344	–	n.a.
9/14/99	1125	WCC57	25346	Turkey	V
9/14/99	1125	WCC57	25348	–	n.a.
9/14/99	1125	WCC57	25349	–	n.a.
9/14/99	1125	WCC57	25347	–	n.a.
9/14/99	1130	WCC58	25350	–	n.a.
9/14/99	1130	WCC58	25351	–	n.a.
9/14/99	1130	WCC58	25352	–	n.a.
9/14/99	1130	WCC58	25353	–	n.a.
9/14/99	1135	WCC59	25356	Turkey	E, V
9/14/99	1135	WCC59	25355	–	n.a.
9/14/99	1135	WCC59	25354	–	n.a.
9/14/99	1135	WCC59	25357	–	n.a.
9/14/99	1140	WCC60	25359	Human	E
9/14/99	1140	WCC60	25360	–	n.a.
9/14/99	1140	WCC60	25361	–	n.a.
9/14/99	1140	WCC60	25358	–	n.a.
9/14/99	1145	WCC61	25363	Avian	E
9/14/99	1145	WCC61	25365	–	n.a.
9/14/99	1145	WCC61	25362	–	n.a.
9/14/99	1145	WCC61	25364	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
10/25/99	1010	WCC62	26318	Avian	n.r.
10/25/99	1010	WCC62	26321	Coyote	E
10/25/99	1010	WCC62	26319	Human	E
10/25/99	1010	WCC62	26320	Turkey	V
10/25/99	1015	WCC63	26324	Avian	n.r.
10/25/99	1015	WCC63	26323	Human	V
10/25/99	1015	WCC63	26322	–	n.a.
10/25/99	1015	WCC63	26325	–	n.a.
10/25/99	1020	WCC64	26328	Avian	n.r.
10/25/99	1020	WCC64	26327	Avian	n.r.
10/25/99	1020	WCC64	26329	Chicken	V
10/25/99	1020	WCC64	26326	–	n.a.
10/25/99	1025	WCC65	26333	Dog	E
10/25/99	1025	WCC65	26332	Human	E, V
10/25/99	1025	WCC65	26330	–	n.a.
10/25/99	1025	WCC65	26331	–	n.a.
10/25/99	1030	WCC66	26335	Avian	E
10/25/99	1030	WCC66	26336	Turkey	V
10/25/99	1030	WCC66	26337	–	n.a.
10/25/99	1030	WCC66	26334	–	n.a.
10/25/99	1035	WCC67	26338	Avian	n.r.
10/25/99	1035	WCC67	26339	Deer	E
10/25/99	1035	WCC67	26340	Opossum	E
10/25/99	1035	WCC67	26341	–	n.a.
10/25/99	1040	WCC68	26343	Avian	n.r.
10/25/99	1040	WCC68	26344	Dog	E
10/25/99	1040	WCC68	26342	Human	E, V
10/25/99	1040	WCC68	26345	Human	E
10/25/99	1045	WCC69A	26346	Turkey	E, V
10/25/99	1045	WCC69A	26349	–	n.a.
10/25/99	1045	WCC69A	26348	–	n.a.
10/25/99	1045	WCC69A	26347	–	n.a.
10/25/99	1045	WCC69B	26352	Avian	n.r.
10/25/99	1045	WCC69B	26351	Dog	E
10/25/99	1045	WCC69B	26353	Poultry	E
10/25/99	1045	WCC69B	26350	Skunk	V
10/25/99	1045	WCC69C	26357	Dog	E, V
10/25/99	1045	WCC69C	26354	Horse	E
10/25/99	1045	WCC69C	26356	Turkey	E, V
10/25/99	1045	WCC69C	26355	–	n.a.
11/1/99	45	WCC71	26366	Horse	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/1/99	45	WCC71	26365	Horse	E
11/1/99	45	WCC71	26367	Human	V
11/1/99	45	WCC71	26368	–	n.a.
11/1/99	900	WCC72	26371	Dog	E
11/1/99	900	WCC72	26370	Duck	V
11/1/99	900	WCC72	26372	Poultry	E
11/1/99	900	WCC72	26369	–	n.a.
11/1/99	1230	WCC73	26374	Deer	E
11/1/99	1230	WCC73	26373	Deer	E
11/1/99	1230	WCC73	26375	Human	E
11/1/99	1230	WCC73	26376	Human	V
11/1/99	1530	WCC74	26378	Horse	E
11/1/99	1530	WCC74	26380	Horse	E
11/1/99	1530	WCC74	26377	Poultry	E
11/1/99	1530	WCC74	26379	Raccoon	V
11/1/99	1630	WCC75	26384	Bovine	E
11/1/99	1630	WCC75	26383	Human	E
11/1/99	1630	WCC75	26381	–	n.a.
11/1/99	1630	WCC75	26382	–	n.a.
11/1/99	1700	WCC76	26386	Bovine	E, V
11/1/99	1700	WCC76	26385	Horse	E
11/1/99	1700	WCC76	26387	Poultry	V
11/1/99	1700	WCC76	26388	–	n.a.
11/1/99	1725	WCC77	26390	Avian	E
11/1/99	1725	WCC77	26391	Bovine	E
11/1/99	1725	WCC77	26389	Dog	V
11/1/99	1725	WCC77	26392	–	n.a.
11/1/99	1800	WCC79	26394	Avian	E
11/1/99	1800	WCC79	26396	Dog	V
11/1/99	1800	WCC79	26395	Human	E
11/1/99	1800	WCC79	26393	–	n.a.
11/1/99	1745	WCC80	26399	Avian	E
11/1/99	1745	WCC80	26397	Avian	E
11/1/99	1745	WCC80	26398	Dog	E
11/1/99	1745	WCC80	26400	–	n.a.
11/1/99	2015	WCC81	26403	Avian	n.r.
11/1/99	2015	WCC81	26401	Human	E
11/1/99	2015	WCC81	26404	Raccoon	E
11/1/99	2015	WCC81	26402	–	n.a.
11/1/99	920	WCC82	26408	Poultry	V
11/1/99	920	WCC82	26406	Poultry	V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/1/99	920	WCC82	26407	Sea Gull	n.r.
11/1/99	920	WCC82	26405	–	n.a.
11/1/99	1400	WCC83	26409	Bovine	E, V
11/1/99	1400	WCC83	26412	Bovine	E, V
11/1/99	1400	WCC83	26410	Dog	E
11/1/99	1400	WCC83	26411	Human	E
11/1/99	1200	WCC84	26436	Avian	E
11/1/99	1200	WCC84	26433	Avian	E
11/1/99	1200	WCC84	26435	Bovine	E, V
11/1/99	1200	WCC84	26434	Dog	E
11/1/99	1225	WCC85	26437	Avian	E
11/1/99	1225	WCC85	26440	Deer	E
11/1/99	1225	WCC85	26438	Human	E
11/1/99	1225	WCC85	26439	Human	E
12/6/99	1045	WCC88	26753	Avian	n.r.
12/6/99	1045	WCC88	26756	Bovine	E
12/6/99	1045	WCC88	26755	Goose	E
12/6/99	1045	WCC88	26754	–	n.a.
12/6/99	1050	WCC89	26758	Avian	E
12/6/99	1050	WCC89	26759	Bovine	n.r.
12/6/99	1050	WCC89	26760	–	n.a.
12/6/99	1050	WCC89	26757	–	n.a.
12/6/99	1055	WCC90	26763	Avian	E
12/6/99	1055	WCC90	26762	Bovine	E, V
12/6/99	1055	WCC90	26761	Horse	E
12/6/99	1055	WCC90	26764	Horse	E
12/6/99	1100	WCC91	26765	Avian	E
12/6/99	1100	WCC91	26768	Bovine	E, V
12/6/99	1100	WCC91	26766	Bovine	E, V
12/6/99	1100	WCC91	26767	Deer	E
12/6/99	1105	WCC92	26769	Human	E
12/6/99	1105	WCC92	26770	–	n.a.
12/6/99	1105	WCC92	26771	–	n.a.
12/6/99	1105	WCC92	26772	–	n.a.
12/6/99	1110	WCC93	26773	Bovine	E
12/6/99	1110	WCC93	26774	Deer	E
12/6/99	1110	WCC93	26775	Feline	E
12/6/99	1110	WCC93	26776	–	n.a.
12/6/99	1115	WCC94	26777	Dog	E
12/6/99	1115	WCC94	26780	Feline	E
12/6/99	1115	WCC94	26779	Sludge	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
12/6/99	1115	WCC94	26778	–	n.a.
12/6/99	1120	WCC95	26784	Bovine	E, V
12/6/99	1120	WCC95	26783	Bovine	E, V
12/6/99	1120	WCC95	26782	Poultry	V
12/6/99	1120	WCC95	26781	–	n.a.
12/6/99	1120	WCC95B	26787	Bovine	E, V
12/6/99	1120	WCC95B	26786	Bovine	E, V
12/6/99	1120	WCC95B	26788	Feline	E
12/6/99	1120	WCC95B	26785	Poultry	V
12/6/99	1120	WCC95C	26789	Avian	n.r.
12/6/99	1120	WCC95C	26791	Bovine	E, V
12/6/99	1120	WCC95C	26792	Bovine	E, V
12/6/99	1120	WCC95C	26790	–	n.a.
1/18/00	1100	WCC96	27132	Turkey	E, V
1/18/00	1100	WCC96	27133	Avian	E
1/18/00	1100	WCC96	27134	Human	E
1/18/00	1100	WCC96	27135	Dog	n.r.
1/18/00	1105	WCC97	27136	Avian	E
1/18/00	1105	WCC97	27137	–	n.a.
1/18/00	1105	WCC97	27138	Bovine	E
1/18/00	1105	WCC97	27139	Avian	E
1/18/00	1110	WCC98	27140	–	n.a.
1/18/00	1110	WCC98	27141	–	n.a.
1/18/00	1110	WCC98	27142	Avian	E
1/18/00	1110	WCC98	27143	–	n.a.
1/18/00	1115	WCC99	27144	–	n.a.
1/18/00	1115	WCC99	27145	–	n.a.
1/18/00	1115	WCC99	27146	–	n.a.
1/18/00	1115	WCC99	27147	Human	n.r.
1/18/00	1120	WCC100	27148	Beaver	V
1/18/00	1120	WCC100	27149	–	n.a.
1/18/00	1120	WCC100	27150	–	n.a.
1/18/00	1120	WCC100	27151	–	n.a.
1/18/00	1125	WCC101	27152	–	n.a.
1/18/00	1125	WCC101	27153	–	n.a.
1/18/00	1125	WCC101	27154	–	n.a.
1/18/00	1125	WCC101	27155	–	n.a.
1/18/00	1130	WCC102	27156	–	n.a.
1/18/00	1130	WCC102	27157	–	n.a.
1/18/00	1130	WCC102	27158	Horse	E
1/18/00	1130	WCC102	27159	Avian	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

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Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
1/18/00	1135	WCC103	27160	–	n.a.
1/18/00	1135	WCC103	27161	–	n.a.
1/18/00	1135	WCC103	27162	–	n.a.
1/18/00	1135	WCC103	27163	–	n.a.
3/20/00	1530	WCC105	27893	Avian	n.r.
3/20/00	1530	WCC105	27894	Raccoon	E
3/20/00	1530	WCC105	27895	–	n.a.
3/20/00	2010	WCC106	27898	Avian	n.r.
3/20/00	2010	WCC106	27897	Poultry	E, V
3/20/00	2010	WCC106	27896	–	n.a.
3/21/00	600	WCC107	27900	Feline	E, V
3/21/00	600	WCC107	27899	Raccoon	E
3/21/00	600	WCC107	27901	Turkey	V
3/21/00	800	WCC108	27903	Avian	n.r.
3/21/00	800	WCC108	27902	Bovine	E
3/21/00	800	WCC108	27904	–	n.a.
3/21/00	950	WCC109	27907	Avian	E
3/21/00	950	WCC109	27905	Horse	E, V
3/21/00	950	WCC109	27906	–	n.a.
3/21/00	1100	WCC110	27908	Human	n.r.
3/21/00	1100	WCC110	27910	–	n.a.
3/21/00	1100	WCC110	27909	–	n.a.
3/21/00	1115	WCC111	27912	Horse	E
3/21/00	1115	WCC111	27913	Human	E
3/21/00	1115	WCC111	27911	–	n.a.
3/21/00	1140	WCC112	27914	Human	n.r.
3/21/00	1140	WCC112	27915	–	n.a.
3/21/00	1140	WCC112	27916	–	n.a.
3/21/00	1335	WCC113	27919	Avian	E
3/21/00	1335	WCC113	27917	Human	n.r.
3/21/00	1335	WCC113	27918	–	n.a.
3/21/00	1815	WCC114	27922	Avian	E
3/21/00	1815	WCC114	27920	Avian	E
3/21/00	1815	WCC114	27921	Feline	E
3/30/00	940	WCC127	28006	Avian	E
3/30/00	940	WCC127	28005	–	n.a.
3/30/00	945	WCC128	28007	Avian	E
3/30/00	945	WCC128	28008	–	n.a.
3/30/00	945	WCC128	28009	–	n.a.
3/30/00	950	WCC129	28011	Avian	E
3/30/00	950	WCC129	28010	Horse	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

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Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
3/30/00	950	WCC129	28012	–	n.a.
3/30/00	955	WCC130	28014	Deer	E
3/30/00	955	WCC130	28013	–	n.a.
3/30/00	955	WCC130	28015	–	n.a.
3/30/00	955	WCC130B	28019	Chicken	E
3/30/00	955	WCC130B	28016	Dog	E
3/30/00	955	WCC130B	28017	Dog	E
3/30/00	955	WCC130C	28020	Deer	E
3/30/00	955	WCC130C	28022	Dog	E
3/30/00	955	WCC130C	28021	–	n.a.
4/24/00	2115	WCC131	29466	Avian	E
4/24/00	2115	WCC131	29467	Dog	E, V
4/24/00	2115	WCC131	29468	–	n.a.
4/25/00	800	WCC134	29471	–	n.a.
4/25/00	800	WCC134	29470	–	n.a.
4/25/00	800	WCC134	29469	–	n.a.
4/25/00	1310	WCC136	29474	Avian	E
4/25/00	1310	WCC136	29473	Septage	n.r.
4/25/00	1310	WCC136	29472	Septage	n.r.
4/25/00	1400	WCC138	29475	Turkey	E
4/25/00	1400	WCC138	29477	–	n.a.
4/25/00	1400	WCC138	29476	–	n.a.
4/25/00	1430	WCC139	29479	Bovine	E, V
4/25/00	1430	WCC139	29480	Bovine	E, V
4/25/00	1430	WCC139	29478	–	n.a.
4/25/00	1500	WCC140	29482	Avian	E
4/25/00	1500	WCC140	29481	Human	V
4/25/00	1500	WCC140	29483	Turkey	V
4/25/00	1530	WCC143	29485	Dog	E
4/25/00	1530	WCC143	29486	Turkey	V
4/25/00	1530	WCC143	29484	–	n.a.
4/25/00	2200	WCC144	29489	Feline	E
4/25/00	2200	WCC144	29488	Poultry	E, V
4/25/00	2200	WCC144	29487	Turkey	E, V
4/25/00	900	WCC145	29490	Raccoon	E
4/25/00	900	WCC145	29491	–	n.a.
4/25/00	900	WCC145	29492	–	n.a.
4/25/00	1230	WCC148	29493	Human	E
4/25/00	1230	WCC148	29495	Septage	n.r.
4/25/00	1230	WCC148	29494	–	n.a.
5/10/00	945	WCC151	30211	Bovine	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

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Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
5/10/00	945	WCC151	30213	Goose	E
5/10/00	945	WCC151	30212	–	n.a.
5/10/00	950	WCC152	30214	–	n.a.
5/10/00	950	WCC152	30215	–	n.a.
5/10/00	950	WCC152	30216	–	n.a.
5/10/00	955	WCC153	30217	Crow	E
5/10/00	955	WCC153	30218	–	n.a.
5/10/00	955	WCC153	30219	–	n.a.
5/10/00	955	WCC153B	30222	Bovine	E
5/10/00	955	WCC153B	30220	Deer	E
5/10/00	955	WCC153B	30221	–	n.a.
5/10/00	955	WCC153C	30225	Bovine	E
5/10/00	955	WCC153C	30224	–	n.a.
5/10/00	955	WCC153C	30223	–	n.a.
6/20/00	1135	WCC156	32012	Bovine	V
6/20/00	1135	WCC156	32011	Dog	E
6/20/00	1135	WCC156	32010	Duck	E
6/20/00	1140	WCC157	32014	Avian	E, V
6/20/00	1140	WCC157	32013	Crow	n.r.
6/20/00	1145	WCC158	32015	Avian	E
6/20/00	1145	WCC158	32016	Human	E
6/20/00	1145	WCC158B	32018	Human	E
6/20/00	1145	WCC158B	32017	–	n.a.
6/20/00	1145	WCC158B	32019	–	n.a.
6/20/00	1145	WCC158C	32021	Avian	E
6/20/00	1145	WCC158C	32022	Feline	E
6/20/00	1145	WCC158C	32020	–	n.a.
6/27/00	1625	WCC160	33106	–	n.a.
6/27/00	1625	WCC160	33104	–	n.a.
6/27/00	1625	WCC160	33105	–	n.a.
6/27/00	852	WCC162	33107	–	n.a.
6/27/00	915	WCC163	33108	Fox	n.r.
6/27/00	915	WCC163	33109	Fox	n.r.
6/27/00	2020	WCC165	33110	Bovine	E
6/27/00	2020	WCC165	33111	Goat	E
6/27/00	2020	WCC165	33112	Human	n.r.
6/27/00	2130	WCC166	33114	Deer	E
6/27/00	2130	WCC166	33113	–	n.a.
6/27/00	2215	WCC167	33115	Avian	E
6/27/00	2215	WCC167	33116	–	n.a.
6/27/00	30	WCC169	33117	Dog	V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

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Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
6/27/00	30	WCC169	33118	Human	E, V
6/27/00	130	WCC170	33119	Bovine	E
6/27/00	130	WCC170	33121	Human	n.r.
6/27/00	130	WCC170	33120	–	n.a.
6/27/00	430	WCC171	33122	Horse	E
8/3/00	820	WCC183	34511	Deer	E
8/3/00	820	WCC183	34509	–	n.a.
8/3/00	820	WCC183	34510	–	n.a.
8/3/00	825	WCC184	34512	Deer	E
8/3/00	825	WCC184	34514	Deer	E
8/3/00	825	WCC184	34513	Deer	E
8/3/00	825	WCC185	34517	Avian	E
8/3/00	825	WCC185	34516	–	n.a.
8/3/00	825	WCC185	34515	–	n.a.
8/3/00	830	WCC186	34520	Horse	E
8/3/00	830	WCC186	34518	–	n.a.
8/3/00	830	WCC186	34519	–	n.a.
8/3/00	835	WCC187	34521	Avian	E
8/3/00	835	WCC187	34523	Bovine	E, V
8/3/00	835	WCC187	34522	–	n.a.
8/3/00	835	WCC188	34524	Avian	E
8/3/00	835	WCC188	34526	Human	E
8/3/00	835	WCC188	34527	Raccoon	n.r.
8/3/00	835	WCC188	34525	Raccoon	n.r.